FERRIHYDRITE AS AN ENTEROSORBENT FOR ARSENIC

A Dissertation

by

JOHN FLOYD TAYLOR

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2010

Major Subject: Toxicology

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Approved by:

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ABSTRACT

Ferrihydrite as an Enterosorbent for Arsenic.

(December 2010)

John Floyd Taylor, B.S., University of Louisiana at Monroe

Chair of Advisory Committee: Dr. Timothy D. Phillips

Arsenic in drinking water is a problem in many developing nations such as Taiwan and Bangladesh. Currently, no oral binding agent exists for the mitigation of arsenic toxicity. The goals of this research were to 1) screen a variety of sorbents for their ability to sorb As from water and screen for potential nutrient interactions with vitamin A (VA) and riboflavin (RF) isotherms; 2) further describe the sorption of As to ferrihydrite using isothermal analysis and a simulated gastrointestinal model (GI), and by testing ferrihydrite's ability to protect Hydra from As toxicity; 3) verify ferrihydrite's safety and efficacy in a short term rodent model.

Ferrihydrite was found to be the most effective sorbent for both As(III) and As(V). Exchanging SWy-2 with sulfur containing organic groups increased the sorption of both As(V) and As(III) compared to the parent clay, though the total As sorbed was much less than As sorption by ferrihydrite.

Ferrihydrite and an industrially produced ferrihydrite (IPF) both sorbed As(V) and As(III) with high capacity. Both ferrihydrites also sorbed As(V) and

As(III) at high capacity in the simulated GI model. Fe measured in the simulated GI tract was below tolerable daily limits for both ferrihydrite and IPF. Ferrihydrite at 0.25% w/w was found to protect Hydra up to 200 times the minimal effective concentration (MEC) for As(III) and over 2.5 times the MEC for As(V), while IPF at 0.25% w/w protected Hydra up to 200 times the MEC for As(III) and just over 2 times the MEC for As(V).

IPF was apparently safe and well tolerated by the rats in our study over a period of 2 weeks. No statistically significant differences were seen in serum biochemistry, serum Fe, serum VA, or serum vitamin E between rats fed control diet versus those fed 0.5% w/w IPF. Ferrihydrite was found to reduce urinary As after a single gavage of 0.5 mL of 500 ppm As(III) or As(V). These results verify in vitro findings and suggest that ferrihydrite is apparently safe and effective as an enterosorbent for As.

DEDICATION

To my wife Beth and unborn child; may I be the husband and father that you deserve, and may my life and ventures from this point forward enrich your lives as well as all those with whom I interact in the future.

ACKNOWLEDGEMENTS

I would like to thank many individuals who have helped me and without whom I would not have been able to complete this dissertation.

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TABLE OF CONTENTS

CHAPTER Page

LIST OF FIGURES

FIGURE Page

FIGURE Page

LIST OF TABLES

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Overview

Arsenic is a toxic and carcinogenic metalloid and is distributed in normally trace quantities within rocks, soils, and bodies of water throughout the world. It exists in tri and pentavalent forms with the most common trivalent forms being arsenic trioxide and sodium arsenite and the most common pentavalent forms being sodium arsenate, arsenic pentoxide, and arsenic acid. Various organic forms of arsenic are important as well including arsenilic acid, arsenosugars, and several methylated forms of arsenic that are metabolic products in animals. The manufacturing of pesticides, herbicides, smelting and production of other agricultural products leads to the bulk of occupational exposures (*1*).

Chronic ingestion of inorganic arsenic from drinking water is the most important human exposure to arsenic. Long term exposure to high enough levels of arsenic leads to characteristic skin lesions, neurological effects, hypertension, cardiovascular disease, pulmonary disease, peripheral vascular disease, and increased incidence of diabetes mellitus. Also, chronic exposure is linked to skin cancers and internal cancers such as bladder, kidney and lung cancers (*2*).

This dissertation follows the style of *Environmental Science and Technology.*

Arsenic contamination of groundwater is a global problem. The sources of arsenic in groundwater are usually natural, though contamination can be caused or exacerbated from As used for industrial purposes, mining activities, metal processing, and application of pesticides and fertilizers containing As. Though the USEPA limit and WHO guideline for As in drinking water is 10 µg/L, contamination of groundwater exceeding this level has been measured in the US in states such as California, Alaska, Arizona, Indiana, Idaho, Nevada, Washington, Missouri, Ohio, Wisconsin, Texas, and New Hampshire (*3-9*). Exposure to even higher levels of As in groundwater occurs in countries such as Bangladesh, India and Taiwan where groundwater contains As levels into the ppm range (*10*).

Current methods for reducing exposure to arsenic in drinking water are either to remediate sources of drinking water or to find alternate sources. Treatments for arsenic exposure include chelating agents with analogs of dimecaprol, oral binders such as charcoal, and potentially, agents that promote methylation of arsenic and its subsequent elimination from the body via urine (*11*). The chelating agent 2,3-dimercaptosuccinic acid was found to be ineffective in a clinical trial focusing on the treatment of chronic arsenicosis (*12*), while non-selective oral binders such as charcoal do not provide a legitimate solution for individuals chronically exposed to arsenic.

The Agency for Toxic Substances and Disease Registry in their recent toxicological profile of As identified “phosphate binders” as a possible treatment

for arsenic ingestion (*11*). The use of these binders, likely iron and aluminum oxides, as enterosorbents has not been investigated previously. Arsenic bound to soil minerals and iron oxides has been shown to have limited bioavailability (*13-15*), which gives some indication that iron oxides or aluminum oxides could be successful enterosorbents of As.

Toxicokinetics of Inorganic Arsenic Ingestion

Inorganic arsenic (either arsenate or arsenite) is absorbed by the gastrointestinal tract at a high percentage (80-90%). It is then distributed throughout the body, methylated, and excreted primarily in the urine. Arsenic tends to migrate to skin, as seen in the characteristic skin lesions it causes, and is also excreted by desquamation of skin and in sweat (Figure 1). Accumulation of arsenic occurs in forming hair and nails. Higher arsenic exposures cause characteristic bands in the fingernails known as Mees' Lines. These symptoms appear about 6 weeks after symptoms of arsenic toxicity appear following an exposure. Arsenic in fingernails and hair have been used as a biomarker for exposure, while urinary arsenic is the preferred biomarker for a current exposure (*1*). Arsenic also interferes with heme synthesis, making an increase in urinary porphyrin excretion a proposed biomarker of exposure as well (*16*).

Inorganic arsenic starts in the form of arsenate (As^{5+}) or arsenite (As^{3+}) (Figure 2). Arsenate is converted to arsenite by arsenate reductase. Arsenite

Figure 1. Characteristic skin lesions caused by chronic exposure to arsenic (2).

methyl transferase then converts arsenite to monomethylarsonic acid (MMA^{5+}). MMA^{5+} is then reduced by arsenic methyltranserase to form monomethylarsonic acid (MMA^{3+}). MMA^{3+} is metabolized to dimethylarsinous acid (DMA^{5+}) and then to dimethylarsinic acid (DMA^{3+}). DMA^{3+} is then further methylated to form trimethylarsenic acid in which As in pentavalent. Though originally the methylation process was thought of as a detoxification mechanism, recent work suggests that the trivalent metabolites MMA^{3+} and DMA^{3+} may be more toxic than the arsenite or arsenate (*17, 18*). Typical urinary profiles of humans exposed to arsenic show that 10-30% is excreted as inorganic arsenic, 10-20% as MMA, and 55-76% as DMA (*1, 18*). Large variations are seen with differences in age and sex, as well as potential differences due to genetic polymorphisms. During pregnancy, higher excretion of DMA has been observed with a decrease in excretion of arsenic as MMA, which may have an impact on the toxicity to the developing fetus (*19*).

Mechanism of As Toxicity

Trivalent arsenic forms are known to react with thiol groups on proteins leading to inhibition of enzymes or altered functions of proteins. Arsenic tends to accumulate in the mitochondria, and a particular target appears to be succinate dehydrogenase. Arsenic inhibits succinate dehydrogenase, which uncouples oxidative phosphorlylation, resulting in decreased ATP production, affecting virtually all cellular functions. Pentavalent arsenic in particular acts as an uncoupler of mitochondrial oxidative phosphorylation, by mimicking phosphate in

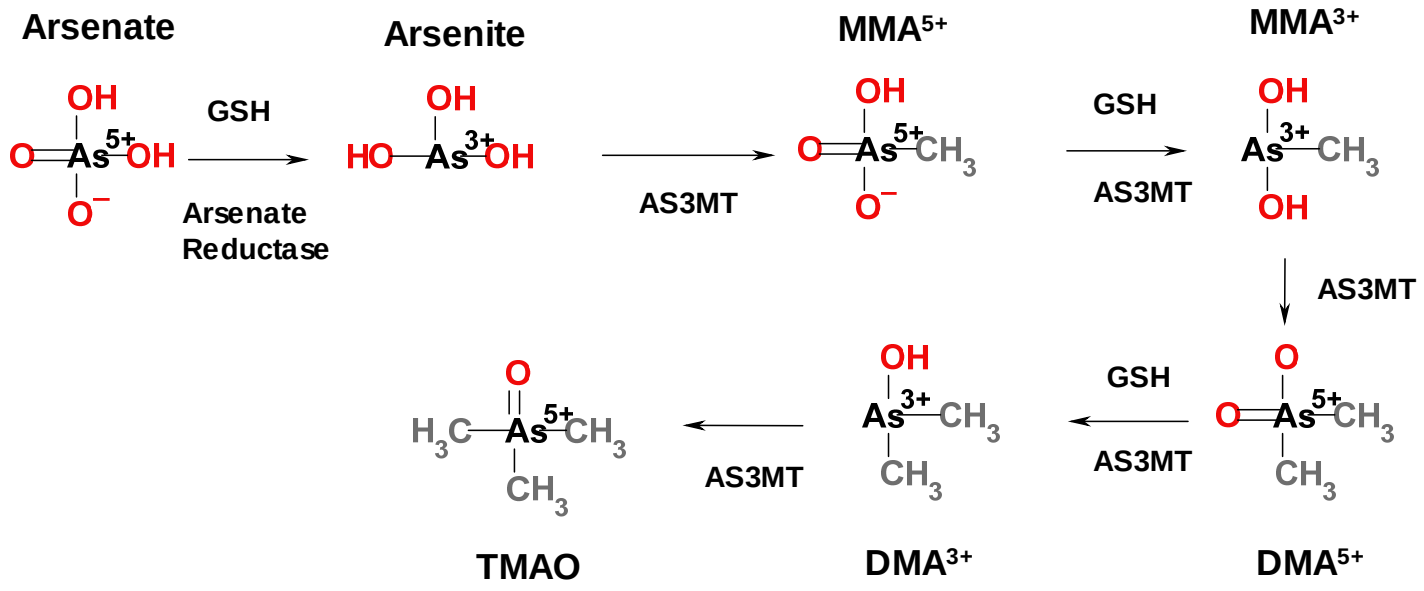

Figure 2. Metabolism of arsenic. Key: GSH, reduced glutathione: AS3MT, arsenic methyltransferase: MMA^{5+}, monomethylarsonic acid: MMA^{3+}, monomethylarsonous acid: DMA^{5+}, dimethylarsinic acid: DMA^{3+}, dimethylarsinous acid: TMAO, trimethylarsenic oxide.

the formation of adenosine triphosphate. Additionally arsenic and its metabolites produce oxidants and resulting oxidative DNA damage, arsenic alters DNA damage repair, and it enhances cell proliferation. Many of these mechanisms along with its ability to produce chromosomal abnormalities likely contribute to its carcinogenicity (*1, 18, 11*). Multiple sources have shown that arsenic is not directly mutagenic (*11*). Mechanisms for cancinogenicity will be discussed in further detail in a later section.

Non Cancer Effects of Chronic Inorganic As Exposure

Skin is a major target organ for chronic ingestion of inorganic arsenic. The first signs are usually diffuse or spotty pigmentation or hypopigmentation with these symptoms usually appearing 6 months to 3 years after the start of the exposure. Long term exposure to arsenic in drinking water leads to a characteristic peripheral vascular disease known “Blackfoot Disease”. Tseng et al. (*11*) reported that this condition was endemic to populations in Taiwan who were exposed to drinking water contaminated with As at levels of 0.17-0.80 ppm. This disease is characterized by endarteritis and gangrene of the lower extremities. Although arsenic induced vascular effects are seen in countries such as Chile, Mexico, India, China, and Bangladesh, they are not as highly affected as the population in Taiwan which suggests other factors are involved in the disease. Table 1 summarizes exposures by geographical region.

Arsenic exhibits other effects on the vascular system besides those manifesting in skin. Exposed populations in Taiwan exhibited an increased

Table 1. Comparison of arsenic in groundwater from selected parts of the world (*10*).

Country/Region	Arsenic Concentration (ppb)	Mechanism of Contamination
Bangladesh	<2-900	Reduction of Fe-oxyhydroxides/Sulfide oxidation in alluvial sediments
West Bengal, India	<1-1300	Reduction of Fe-oxyhydroxides/Sulfide oxidation in alluvial sediments
China, Xinjang	<50-1860	Reducing environment in alluvial sediments
Taiwan	up to 1820	Oxidation of pyrite in mine tailings
Thailand	120-6700	Oxidation of mine wastes and tailings
Argentina	100-4800	Volcanic Ash with 90% rhyolitic glass
Mexico, Zimapan	300-1100	Oxidation of sulfide from mine wastes
Hungary	25-50	Complexation of As with humic substances
USA	100-500	Desorption of As from Fe-oxyhydroxides/sulfide oxidation
Canada, Nova Scotia	18-146	Oxidation of sulfides
UK	>10	Oxidation of sulfides from mine wastes

incidence of cerebrovascular and microvascular diseases (*20, 21*) and ischemic heart disease (*22-26*). Arsenic exposure has been associated with an increase in hypertension in Bangladesh (*27*). An autopsy of five children from Chile who died of apparent arsenic toxicity exhibited a thickening of small andmedium sized arteries in tissues throughout the body, particularly the heart (*28*).

Chronic arsenic exposure affects the liver first as jaundice then manifests as abdominal pain, and hepatomegaly. Injury can further progress to cirrhosis and ultimately hepatocellular carcinoma (*1*). Clinical manifestations are usually swollen and tender liver and elevation of liver enzymes. Although effects are most common in doses ranging from 0.01-0.1 mg As/kg/day, effects have been seen in individuals chronically exposed to as little as 0.006 mg/kg/day (*11*).

Chronic exposure to inorganic arsenic can lead to peripheral neuropathy. The first symptoms are numbness of the extremities, but it can lead to painful conditions where the hands and feet develop a “pins and needles” sensation (*1, 11*). Sensory and motor nerves can be affected, with accompanied muscle tenderness, weakness, and progression from proximal to distal muscle groups. Histological examination of nerves from affected individuals reveals a dying-back axonopathy with demyelination. Recovery is seen after the exposure is removed, but is a slow, usually incomplete process. Neurological effects are typically not seen in individuals exposed to doses of 0.0006 mg/kg/day, however fatigue, headache, dizziness, insomnia, nightmares and numbness of the

extremities were reported in a Chinese population exposed to 0.0005 mg/kg/day (*11*).

Several studies have shown an association between chronic exposure to inorganic arsenic and increased incidence of diabetes mellitus (*1, 11*). Autopsies of 5 children who died from chronic exposure to arsenic revealed arterial thickening of the pancreas (*11*) while rats exposed to arsenic trioxide at 2.3 mg/kg/day showed a decrease in the number of islet cells in the pancreas. The rats also displayed reductions in pancreatic superoxide dismutase, pancreatic catalase with increases in nitric oxide and malondialdehyde (*11*).

Chronic arsenic ingestion from drinking water has been associated with numerous developmental effects. Exposure has been associated with excess incidence of miscarriages, stillbirths, preterm births, and infants with low birth weights in Bangladesh, India, and Taiwan (*29- 31*). Similar associations have been made between late fetal mortality, neonatal mortality, and postneonatal mortality and exposure to high levels of arsenic in the drinking water, based on comparisons between subjects in low- and high-arsenic areas of Chile (*32*). In animals, numerous studies have reported developmental effects at arsenic levels that also exhibit overt toxicity to the mother (*11*).

Possible Mechanisms of As Induced Cancers

IARC (2004) has classified arsenic as a known human carcinogen as it is associated with tumors of the skin, lung, bladder, kidney, liver, and prostate (*33*). The mechanism of cancer formation is somewhat unclear, and it has been

difficult to confirm using animal models (*1*). Arsenic has, however, been shown to be a complete transplacental carcinogen in mice (*11, 34-37)*. Arsenic does not acutely induce point mutations, but delayed mutagenesis, oxidative DNA mutations, chromosomal aberrations, and sister chromatid exchanges have been reported. Arsenic may act by potentiating mutagenicity observed with other chemicals (*11*). This effect may be the result of direct interference with DNA repair processes, by inhibiting DNA ligase (*17*) or other DNA repair enzymes (*11)*. Arsenic also induces DNA amplification (*38*). Several authors have hypothesized that methylation changes in genes or their control regions can lead to altered gene expression, and potentially, carcinogenesis (*11*). A study of exposed humans in Taiwan showed that individuals with lower secondary methylation indices, as indicated by the ratio of DMA to MMA in the urine, have an increased risk of bladder cancer (*39*) particularly those highly exposed. Arsenite exposure in a human lung adenocarcinoma cell line resulted in hypermethylation of cytosine in the promoter region of p53, an important tumor suppressor gene (*40*). Inorganic arsenic was shown to upregulate NF-Kb at low concentrations which lead to proliferation of human karatinocytes (*41*). In a mouse model, arsenic was shown to be a co-mutagen with UV-radiation (*1*). Lui et al. showed that in utero exposure of mice resulted in arsenic-altered gene expression including activation of oncogenes and HCC biomarkers, and increased expression of cell proliferation-related genes, stress proteins, and insulin-like growth factors and genes involved in cell-cell communications (*42*).

Epidemiological Studies of As Induced Cancers

Increased incidence of skin cancer due to inorganic arsenic exposure has been reported from a number of studies. Lesions commonly observed include multiple squamous cell carcinomas, which can appear from hyperkeratotic warts or corns (*11*). A study from Tseng et al. was long used as a model study for the evaluation of skin cancer risk from arsenic exposure (*43*). This study used a very large sample size (>40,000), along with inclusions of males and females in a lifetime study. From this study the EPA calculated the upper-bound excess lifetime cancer risk from exposure to 1 µg As/L at $5x10^{-5}$ (*11*).

Besides skin cancer, many studies exist linking As exposure via drinking water to internal cancers. Chen et al. reported odds ratios for developing bladder, lung and liver cancers of 3.90, 3.39, and 2.67, respectively, when comparing those drinking As contaminated artesian well water versus those not drinking contaminated water (*44*). Numerous other large scale epidemiological studies have reported similar results with associations and or dose response trends for bladder, kidney, liver, lung and prostate tumors (*11*). Even though the guideline for arsenic in water was set at 10 ppb by WHO, they have stated that in reality the limit should be set at 0.17 ppb to protect human health and prevent excess cancers (*11*).

Methods to Treat As Exposure

Current methods to treat arsenic exposure include chelating agents, often those that include sulfhydryl groups. These chelating agents act by potentially

binding free As, thus reducing biologically active As. Agents used include dimerceprol (BAL), D-penacillamin, 2,3-dimercaptosuccinic acid, dimercaptosuccinic acid (DMSA), dimercaptopropyl phthalamadic acid (DMPA), and dimercaptopropane sulfonic acid (DMPS) and N-acetylcysteine (*11*). Chelation therapy should be used with caution, however, as side effects such as pain, fever, hypotention, and nephrotoxicity have been reported (*45*). Although there are reports showing success for treatment of accidental poisonings (*46, 47*), studies on the use of chelating agents for the prevention of toxicity from chronic exposure have been less promising. A randomized placebo trial of 2,3-dimercaptosuccinic acid as a therapy for chronic arsenosis due to drinking contaminated water found no significant difference between patients treated with 2,3-dimercaptosuccinic acid and those treated with a placebo (*48*). Recently, ATSDR suggested that metal oxides or “phosphate binders” required study for their ability to potentially protect people from As (*11*).

Iron Oxides

The oxides, hydroxides and oxyhydroxides of Fe (hereafter referred to generally as Fe oxides) are important components of many natural and man-made systems. These minerals have strong pigmenting power, with small quantities leading to vivid red, yellow, orange, brown, and blue-green colors in soils. This makes a simple color examination a useful tool to augment other identifications methods such as X-ray diffraction and infrared spectroscopy. Fe oxides found naturally usually occur as small crystals (5-100 nm), and they have

reactive surfaces capable of sorbing a wide range of both inorganic and organic species. The uses of iron oxides include pigments, jewelry, catalysts, pharmaceuticals, abrasives, sorbents for water and air purification, components of animal feeds, automobile air bags, magnetic tapes, and raw materials for the iron and steel industry (*49*).

A total of 15 iron oxides have been described, of which 12 are known to occur in nature. The naturally occurring Fe oxides include the oxides hematite, magnetite and maghemite, the hydroxides ferrihydrite and green rust, and the oxyhydroxides goethite, lepidocrocite, and schwertmannite (Figure 3). Of these, the most common is goethite (*49*).

Goethites produced for research usually are acicular crystals with a total surface area 20-80m^2/g depending on the method used to produce them. Goethite's structure consists of $FeO_3(OH)_3$ in octahedral coordination linked by corner sharing with hydrogen bridges (*50*). Goethite is found in almost every type of surface environment either alone, or in association with one or more of the Fe oxides (*49*). It is found in greater amounts in cool, wet climates with elevated organic matter.

Hematite is another thermodynamically stable Fe oxide that is abundant in nature. It is typical in the aerobic soils of the tropics, subtropics, arid and semiarid zones and Mediterranian climates. It is typically found in areas that are warmer and with lower organic matter than is preferred for goethite formation

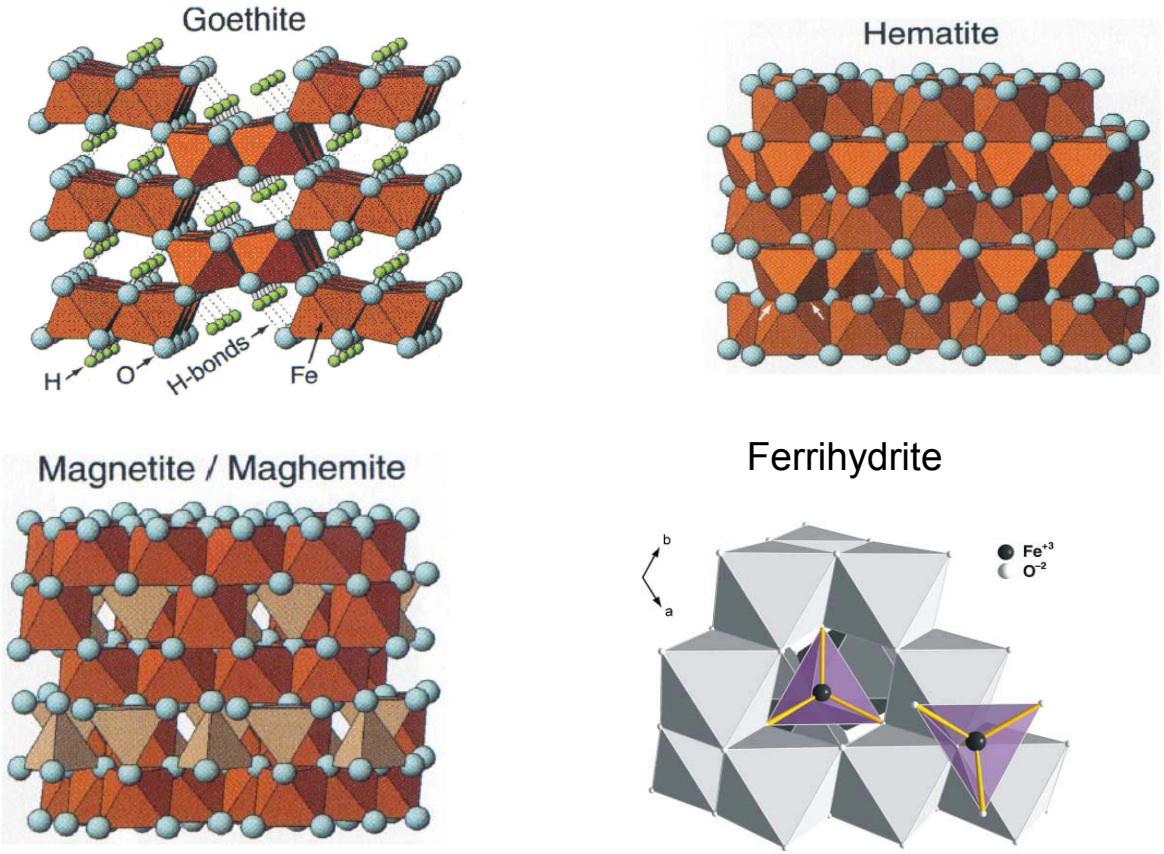

Figure 3. Polyhedral representations of the structures of common iron oxides. Goethite, hematite and magnetite/maghemite are adapted from (*49*) while ferrihydrite's structure is adapted from (*51*).

(*49*). Hematite consists of layers of FeO_6 octahedra which are connected by edge- and face-sharing. Two thirds of its octahedral interstices are filled with Fe(III). Synthetic hematites described in Shwertman and Cornell (2000) have a surface area between 30 and 90 m^2 /g (*50*).

Ferrihydrite is a poorly ordered Fe(III) mineral with a very high surface area due to its poor crystallinity. Its surface area is usually measured between 200-320 m^2/g, although its theoretical surface area is around 600 m^2/g (*49, 50*). For a long time it was believed that its structure was similar to that of hematite, although recent work shows that it contains some tetrahedrally coordinated Fe(III) along with predominantly octahedrally coordinated Fe (*51*). In the lab, 2 and 6 line ferrihydrite, which refers to the number of peaks in a typical X-ray diffraction pattern, are usually the product of rapid hydrolysis of Fe(III) solutions at near neutral pH's (*50*). However, in nature, ferrihydrite is typical of young soils or a product when Fe(II) in solution is rapidly oxidized in the presence of crystallization inhibitors such as organic matter (*49*). As it is metastable, ferrihydrite will recrystalize to form goethite or hematite if left in suspension with water (*50*). Because it also contains some structural water, recrystalization to hematite and goethite has been observed in ferrihydrites stored as a powder (*50*).

Magnetite and maghemite are both magnetic minerals that are commonly found in soils. Both Fe oxides have a cubic structure with 1/3 of the interstices tetrahedrally coordinated and 2/3 in octahedral coordination. In magnetite,

roughly half the positions in octahedral coordination are filled with Fe(II), whereas maghemite is completely oxidized Fe(III). Magnetite is typically inherited from the parent rocks, although it may form as a result of bacterial processes that use Fe as an electron acceptor for respiration. Maghemite is usually formed either by oxidation of magnetite or under heat in the presence of organic matter. The latter pathway leads maghemite to be a common soil component in the weathered soils of the tropics (*50*). Although their surface areas are typically low compared to ferrihydrite, their magnetic properties provide interesting application as sorbents due to their ease of removal from solution.

Basic Makeup of Silicates

The silicate mineral class is very large, and silicates constitute well over 90% of the earth's crust and the bulk of most soils as well. They occur as either primary minerals derived from their parent rocks or as weathering products of primary minerals. The basic building block of silicates is the SiO_4 tetrahedron, with individual tetrahedra linked together by sharing O^{2-} ions to form more complex structures. Another common configuration is the octahedron where 6 0^{2-} ions surround a central cation.

The ability of an atom to occupy a tetrahedral or octahedral site is determined by its atomic radius. The central void in a tetrahedron is smaller than that of an octahedron with the theoretical maximum atomic radius being 0.032 nm for a tetrahedron. In nature, larger cations such as Al^{3+} at 0.54 nm,

and Fe^{3+} at 0.065 nm can also occupy the tetrahedral positions in minerals. The most common element in tetrahedral coordination is Si, which has an atomic radius of 0.026 nm. Common cations in octahedral coordination in minerals include Al^{3+}, Mg^{2+}, Fe^{2+}, Fe^{3+}, and Mn^{2+} (*49*) (Table 2).

The Tetrahedral Sheet

The tetrahedral sheet consists of SiO_4 tetrahedra arranged such that the O^{2-} ions of each tetrahedron are shared with three nearest neighbor tetrahedral (Figure 4). All the shared O^{2-} ions are on the same plane with these ions being referred to as basal oxygens. Two adjacent tetrahedral share only one O^{2-} between them. The fourth O^{2-} ion of each tetrahedron is free to bond to other polyhedral elements. These O^{2-} are called apical oxygens. Each basal oxygen contributes -1 charge to each Si^{4+}, resulting in a neutral tetrahedral sheet.

The Octahedral Sheet

There are two ways to fill the space of OH^- ions in hexagonal closest packing. Divalaent cations such as Mg^{2+} can be included into each octahedral site leading to all three octahedral sites filled, thus the arrangement is called trioctahedral. Conversely, if a mineral or octahedral sheet has trivalent cation such as Al^{3+}, only two trivalent cations are needed for charge neutrality. This arrangement is called dioctahedral (Figure 5).

Table 2. Ionic radii of common elements found in phylosillicate minerals.

Ion	Ionic Radius (nm)[a]	Typical Coordination
Si^{4+}	0.026(4[b])	Tetrahedral
Al^{3+}	0.054(6)	Tetrahedral and Octahedral
Fe^{3+}	0.061(6)	Tetrahedral and Octahedral
Mg^{2+}	0.065(6)	Octahedral
Fe^{2+}	0.078(6)	Octahedral

[a] Ionic radii vary with coordination number
[b] Coordination number

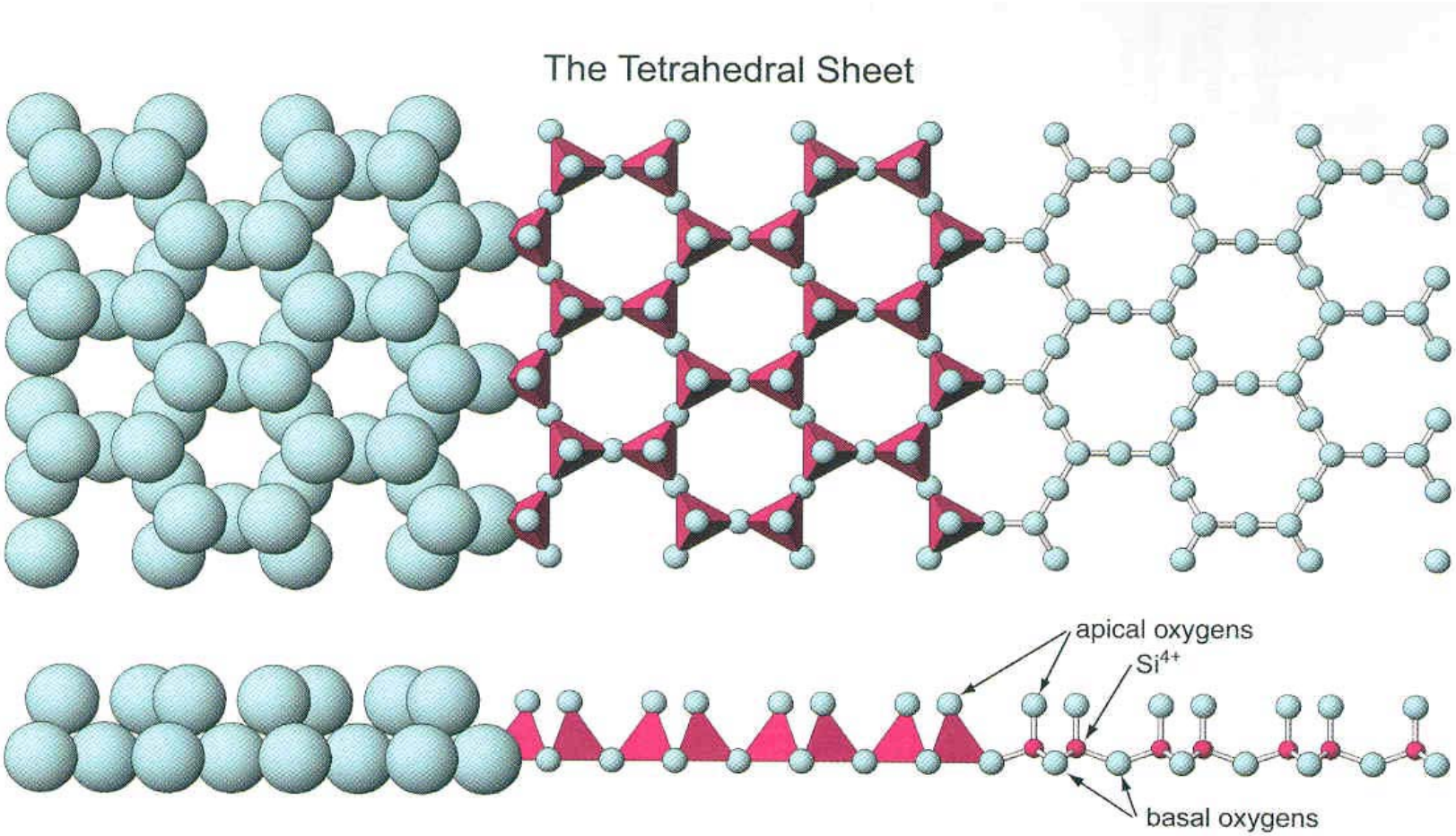

Figure 4. Top and side views of the tetrahedral sheet in minerals. Adapted from (*49*).

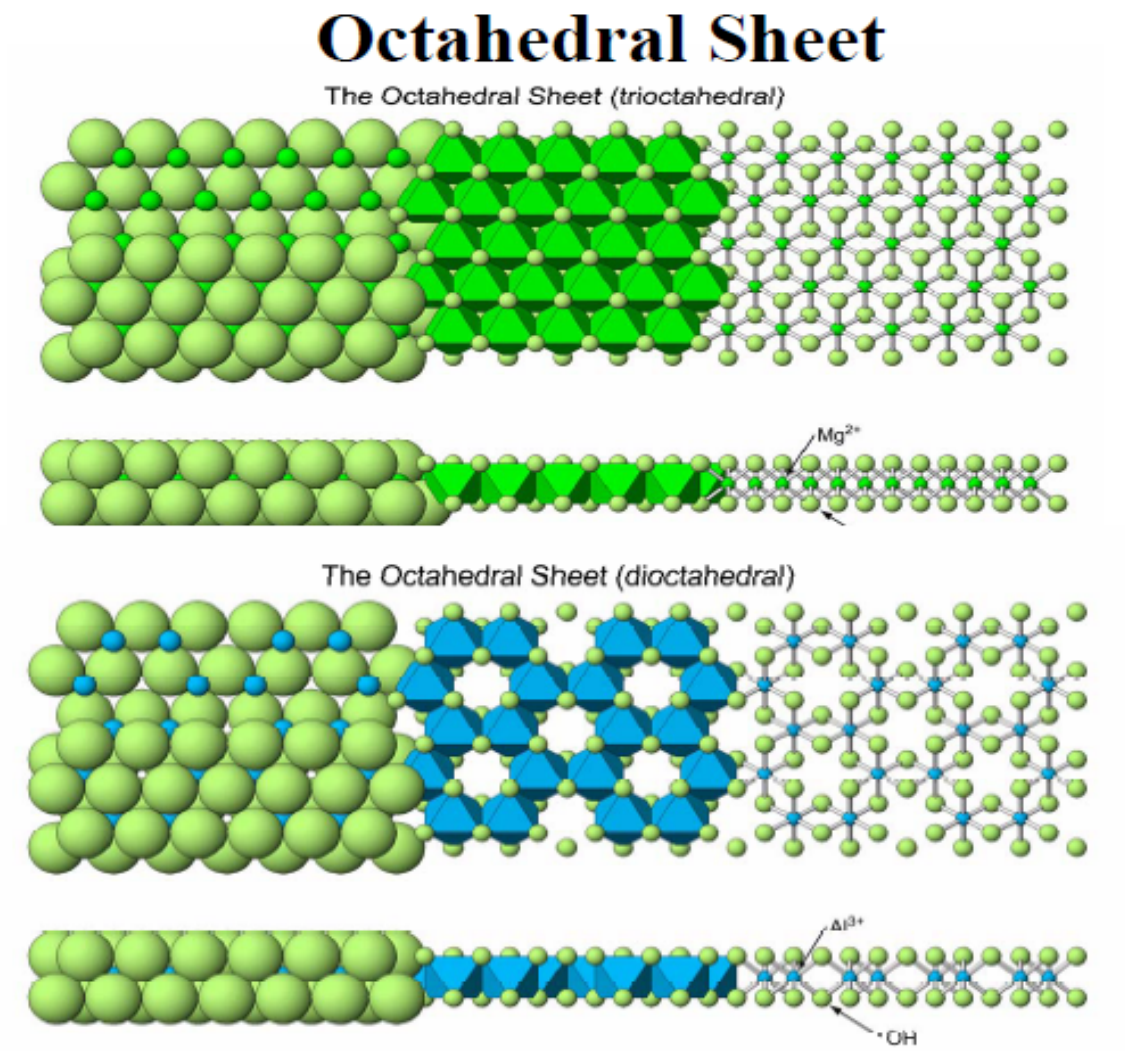

Figure 5. Three dimensional representation of tri- and dioctahedral sheets. Adapted from (*49*).

Phyllosilicate Minerals

Of the silicate classes the phyllosilicates are the ones most common in the clay (<2 μm) fraction of soils. They generally have high surface areas and important cation exchange properties. The composition of phyllosilicates has two basic components, the octahedral and tetrahedral sheet with the layers connecting by sharing apical O^{2-} ions. For charge neutrality a tetravalent cation (usually Si^{4+}) occupies tetrahedrally coordinated sites, while a trivalent or divalent cation occupies octahedrally coordinated layers in the case of dioctahedral and trioctahedral sheets, respectively. However, atoms with similarly sized radii may substitute for the predominant cation in a tetrahedral or octahedral layer. This process is called isomorphic substitution. This leads to the layers of phyllosilicate minerals having a permanent negative charge that is offset by binding other cations to balance the charge. The measurement of the total cations that are needed to balance the negative charge is called cation exchange capacity. The amount of isomorphic substitution and location within the mineral framework can influence the total cation exchange capacity and how strongly cations are attracted to the surface (*49*).

1:1 Minerals

The structure of 1:1 minerals consists of one tetrahedral sheet and one octahedral sheet which share apical O^{2-} ions with part of the octahedral sheet. In looking at a cross section of a 1:1 mineral the basal O's of the tetrahedral

layer are exposed and the OH's make up the other surface (often depicted as the "bottom"). In kaolinite, the most common 1:1 mineral in soils, the layers of the mineral are arranged such that the octahedral layer OHs are hydrogen bonded to the next layers tetrahedral O's. Kaolinite typically has no isomorphic substitution, leading to a low CEC along with a small surface area (*52*). Surface areas for halloysite, which has a similar structure to kaolinite except the 1:1 layers are separated by a layer of water, are somewhat higher (*49*) (Figure 6). Although the 1:1 minerals are not usually the most desirable mineral sorbents, they occur along with other desired minerals for sorbents such as those added to animal feeds (*53*).

2:1 Minerals

The structure of 2:1 minerals consists of two tetrahedral layers bound to each side of an octahedral sheet. Therefore the external surface of the minerals are the basal oxygens of the tetrahedral sheets. The site of isomorphic substitution, i.e. octahedral or tetrahedral, and the amount of internal charge deficit, and overall CEC is important to the behavior of 2:1 minerals. When substitution occurs in the tetrahedral layer it leads to a charge deficit that is shared by the neighboring 3 oxygens, whereas in the case of substitution in the octahedral

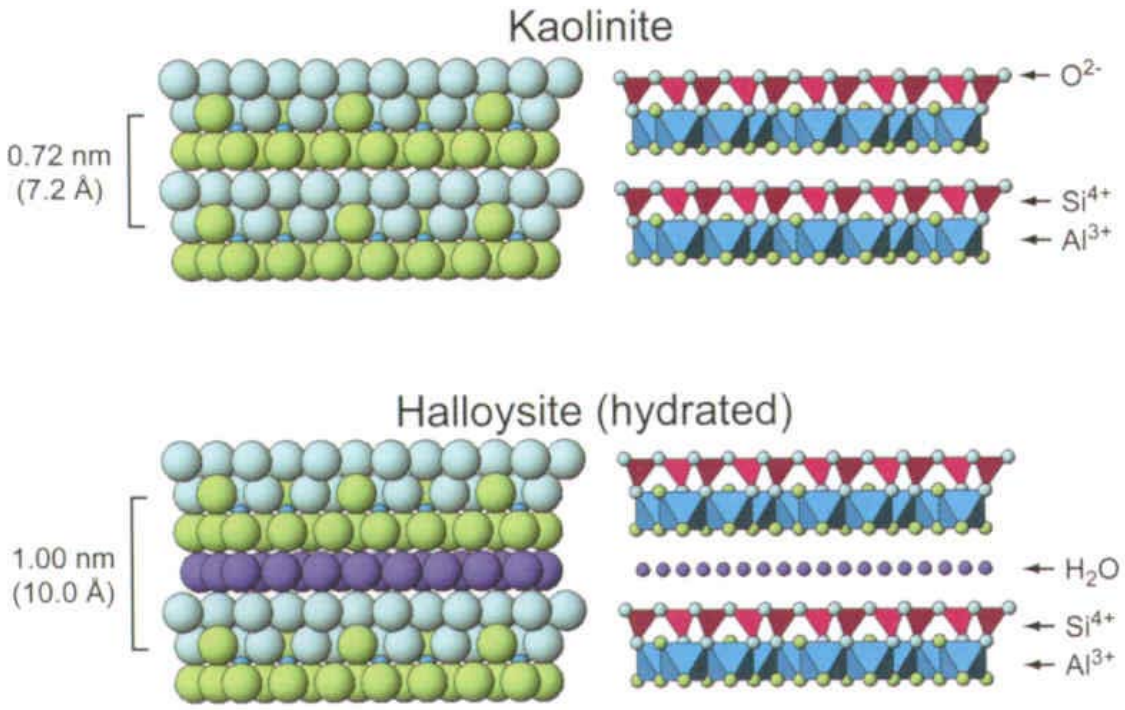

Figure 6. Structural scheme of the two most common 1:1 minerals, kaolinite and halloysite. Adapted from (*49*).

layer, the charge is dispersed over a larger portion, thought to be 9 oxygens (*52*), of the structure which allows the charge to be more dispersed.

Common 2:1 Minerals

Talc and pyrophyllite are the two most basic 2:1 minerals. Talc has Mg^{2+} in the octahedral layer and is trioctahedral, while pyrophyllite is dioctahedral with Al^{3+} occupying sites in the octahedral layer. Very little isomorphic substitution occurs in either, leading to low CEC's. Adjacent layers are held together by weak van der Waals forces.

Micas have the same 2:1 layer structure as talc and pyrophyllite, but they typically have Al^{3+} substituted for Si^{4+} in the tetrahedral layer (Figure 6). Mica typically has a negative charge per formula unit of 1. This high charge combined with the deficit location of the tetrahedral layer leads the mineral to "fix" monovalent cations (usually K^+) in the interlayer of the mineral. Thus the CECs of micas are relatively low (5-15 $cmol^+ / kg^{-1}$) as is the total surface area. Like kaolintes, although micas are not usually effective sorbents, but are a component of soils often found with more effective sorbents such as smectites.

Vermiculites are similar to micas, with the exception of only having a per unit charge of 0.9-0.6. They contain exchangeable cations such as Ca and Mg in the interlayer that are hydrated. This high charge per unit formula gives vermiculites a high CEC while also giving it an affinity for weakly hydrated cations such as K^+, NH_4^+, and Cs^+. This high layer charge leads to vermiculite having a tendency to "fix" potassium, similarly to micas. This tendency leads to

the interlayer spacing of vermiculites being less than what is observed for smectites when hydrated cations are present. Vermiculites also have a much lower tendency to swell than smectites.

Smectites are 2:1 minerals that have a layer charge of 0.6 to 0.25 per formula unit (Figure 8). Smectites swell far more readily than vermiculites, with this being responsible for the shrink-swell behavior characteristic of Vertisols. The three most common minerals in of the smectites group include montmorillonite, beidellite and nontronite (Table 3). Smectites have very high surface areas and adsorptive capacities. They are also used as catalysts, adsorbents for spills, cat litter, sealants for ponds, in drilling fluids for oil wells, and in liners for landfills (*49*).

Zeolites

Another important mineral group is the zeolites. These minerals consists of SiO_4 tetrahedrans arranged such that there are large amounts of pore space within the crystal structure. Aluminum can substitute for Si, leading to a large net negative charge that is balanced by cations. Small cations can move freely inside the porous structure while larger cations are excluding leading zeolites to be called molecular sieves. They are rare in natural soils as they weather easily. Zeolites are widely used in industry and agriculture as catalysts, additives to animal feeds and as ingredients in laundry detergents (*49*).

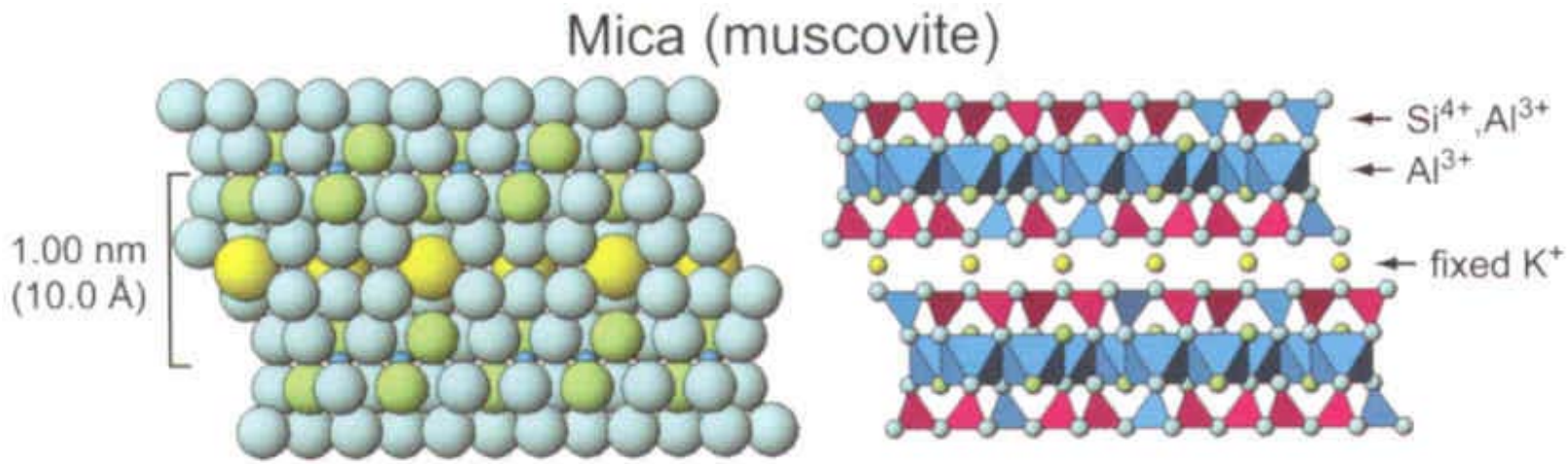

Figure 7. Structural scheme of muscovite. Adapted from (*49*).

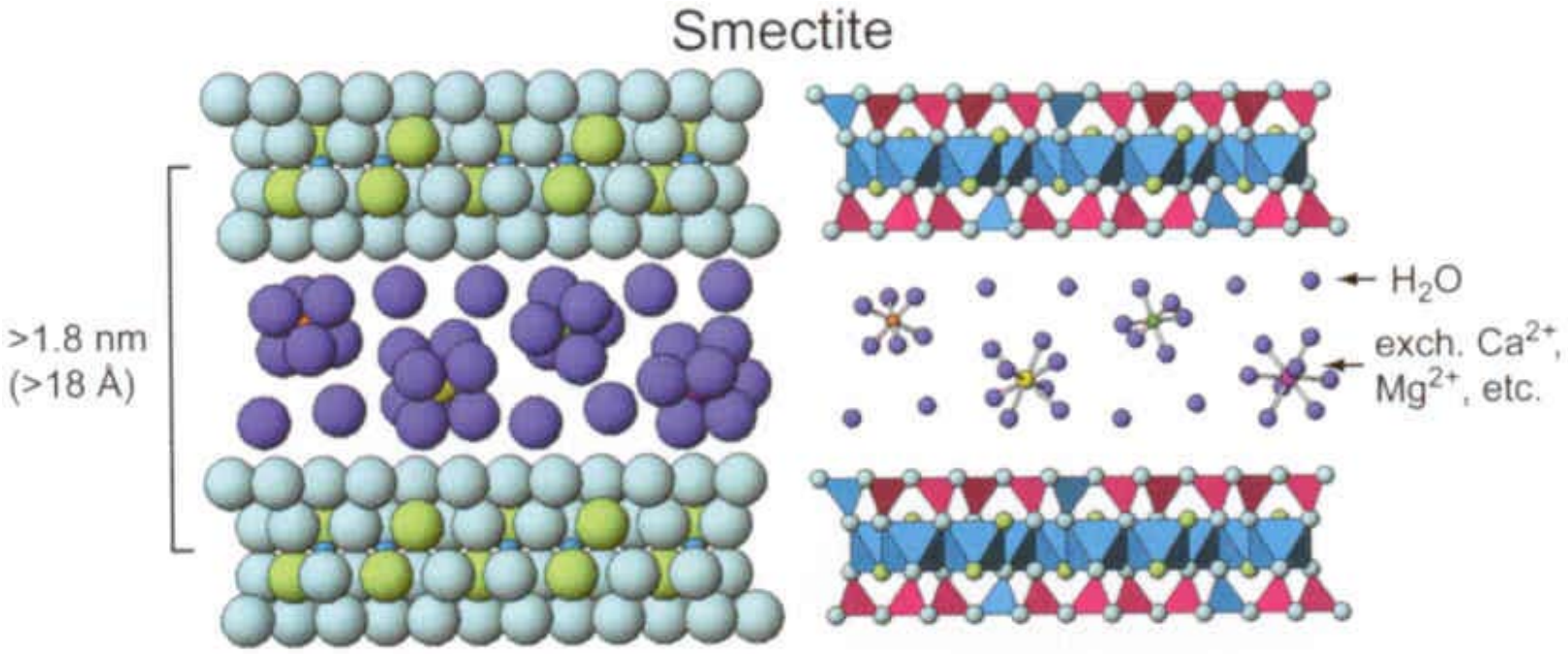

Figure 8. Structural scheme of smectite. Adapted from (*49*).

Table 3. Classification of 2:1 layer silicate minerals.

Mineral Group	Layer Charge per Formula Unit In		Predominant Octahedral Cation	
	Tetrahedral Sheet	Octahedral Sheet	Al^{3+} (Dioctahedral)	Mg^{2+} (trioctahedral)
Pyrophyllite-Talc	0	0	Pyrophyllite	Talc
Smectites	0.25-0.6	0	Beidellite	Saponite
	0	0.25-0.6	Montmorillonite	Hectorite
Vermiculites	0.6 to 0.9	0	Vermiculite	Vermiculite
Micas	1	0	Muscovite	Biotite

Sorption of As by Oxides and Soil Minerals

The reactive elements of oxides and soil minerals for As and anions in general are conditionally charged hydroxyl groups at the surface of the minerals (Figure 9). These arise at the edge sites of clay minerals and oxides because terminal OH groups can be undercoordinated, which means that they do not share enough bonds with neighboring cations to be charge balanced. This leads to OH groups having a charge of ±0.5 and a pH dependent charge depending on whether the OH picks up a proton or not. These sites are more prevalent on oxide minerals, as Fe or Al oxides can contribute the bulk of conditionally charged OH groups in a bulk soil, even though they may only represent a small fraction of the total soil (*49*).

Arsenite in solution exists as H_3AsO_3 and has a pK_a1 of 9.2 and pK_a2 of 12.7 while arsenate exists as H_3AsO_4 with a pK_a1 of 2.3, pK_a2 of 6.8, and pK_a3 of 11.6 (*54*). Both oxidation states of As are commonly found in reduced and oxidized environments due to slow redox transformations (*55*). pH has a marked influence on the binding of both arsenite and arsenate with preferred pH ranges for arsenate sorption typically falling between 3 and 7, while preferred pH ranges for arsenite sorption are typically 7 to 9 (*54*). Arsenite and arsenate form inner sphere complexes on the surfaces the various minerals as detailed from spectroscopic studies (Figure 10). Poorly crystalline Fe and Al oxides show the highest capacity for both arsenate and arsenite adsorption due to higher

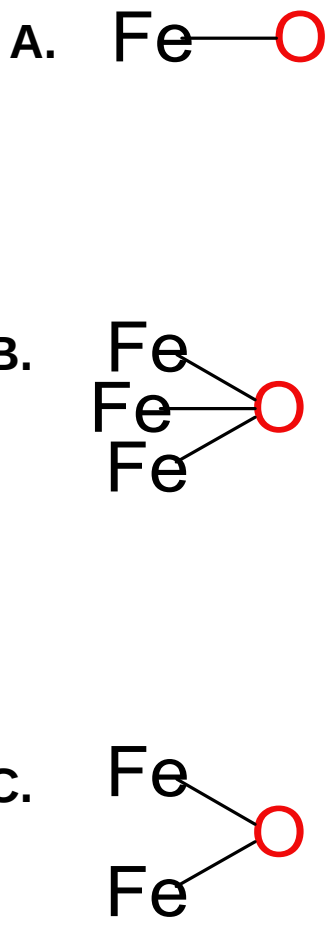

Figure 9. A, B and C type surface oxygens on iron oxides. Iron contributes +0.5 charge to each oxygen (-2) on the surface with the remaining charges balanced by H which contributes +1. In the case of A and B type oxygens, this leads to a pH dependent charge of ± 0.5.

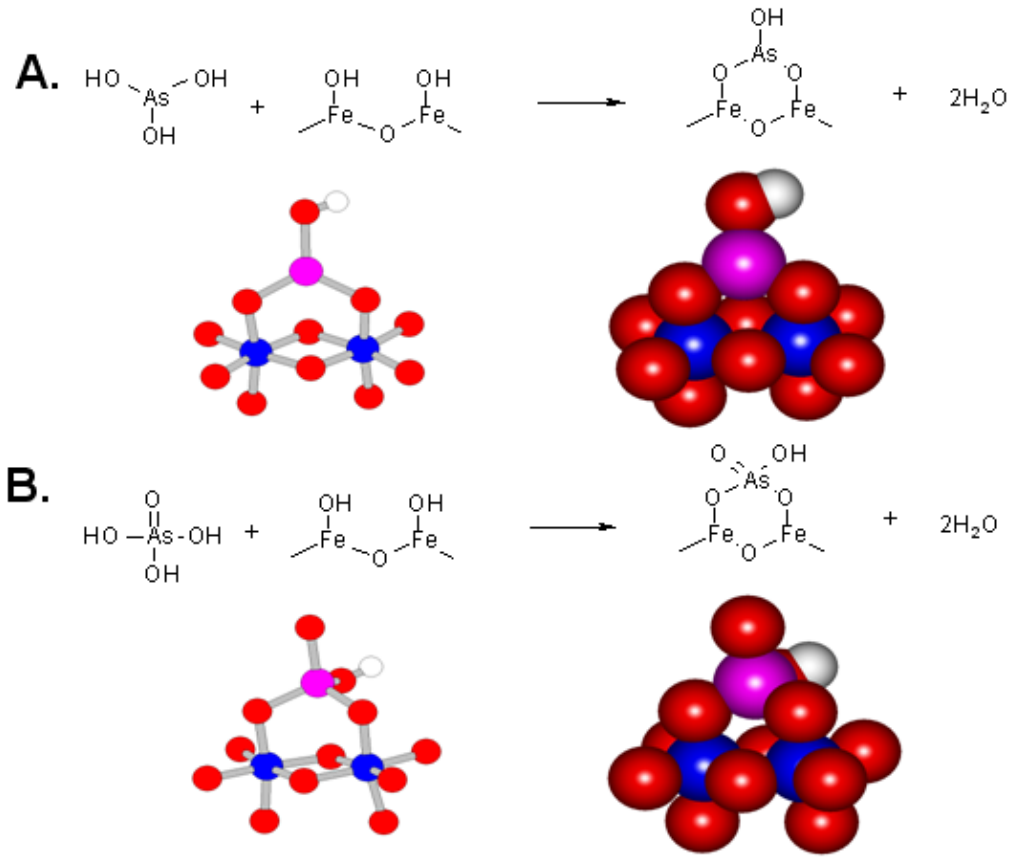

Figure 10. Schematic and molecular models of binuclear bidentate binding of arsenite (A) and arsenate (B) on the surface of ferrihydrite.

concentrations of reactive OH groups on their surfaces. Ferrihydrite, however, was shown to sorb both arsenate and arsenite better at lower pH (2.5) than amorphous Al oxide (*54*). The As binding reaction with ferrihydrite is pH dependent with As(V) being adsorbed in larger quantities at lower pHs, and As(III) at higher pHs. The two species are equally adsorbed at approximately pH 6-7.5 (*56*). At higher pHs, As(V) was found to steadily decrease in amount adsorbed while As(III) bound remained high at increasing pH's up to about 10 (*56*).

The binding of arsenic to Fe and Al oxides is one of the leading methods for treating water contaminated with As. They are effective over the pH range found in most water (*54*). Iron oxide coated sand and columns with zero valent iron have been used to pack columns to treat water, but the same basic reaction with the oxide surface is responsible for sorption of As (*57, 58*). Zero valent iron produces more surface area for sorption as the material oxides. Simple filters using iron scraps, which increase in As sorption as they oxidize, have been constructed and implemented for communities in developing nations (*59*).

Bioavailability of As From Soil

Incidental consumption of soil has been noted as a potential source of As exposure, particularly in children (*60-62*). Currently the EPA uses complete soil digests to assess As contamination and assumes 100% bioavailability of the As present (*63*). Recent research, however, suggests this is not the case. As contaminated soil from pesticide use was analyzed using a rat model, and only a maxiumum of 9.87% of As^{3+} was bioavailable, while only a max of 2.98% of As^{5+}

was bioavailable (*64*). A study in monkeys showed that relative bioavailability (RBA) of As from contaminated soils was inversely correlated with iron sulfate minerals in the soil. Many soil types were included in these experiments and the RBA's were generally less than 30% (*13*). Yang et al found that bioavailablity of As from soils was negatively correlated with iron oxide content and pH. (*65*). A study measuring bioavailability of arsenate loaded ferrihydrite using an in vitro assay found that over 95% of the arsenate remained bound throughout both the simulated stomach and intestine (*66*). These studies and others show the importance of soil sorption of As on its bioavailability and give an indication that certain soil minerals and oxides may be used as enterosorbents for the prevention of As induced toxicity.

Enterosorption Therapy for Aflatoxin

The aflatoxins are a group of mycotoxins produced by the fungi *Aspergillus flavus* and *A. parasiticus*. Aflatoxin B1, the most toxic congener, is a potent liver toxin and liver carcinogen. The molds grow on common foods such as maize, groundnuts and cottonseed. Mold growth, and thus mycotoxin production is exacerbated by drought (*67*). Aflatoxin from effected food is a problem in food grown throughout the temperate and tropical regions of the world, with latitudes between 40°N and 40° designated as the so called "hot zone" for aflatoxin production (*68*).

NovaSil (NS) clay, a calcium montmorillonite, has been identified as a high affinity, high capacity sorbent of aflatoxins (*69*). It has shown effectiveness in vitro, and has been shown to be safe and effective when used as enterosorbent

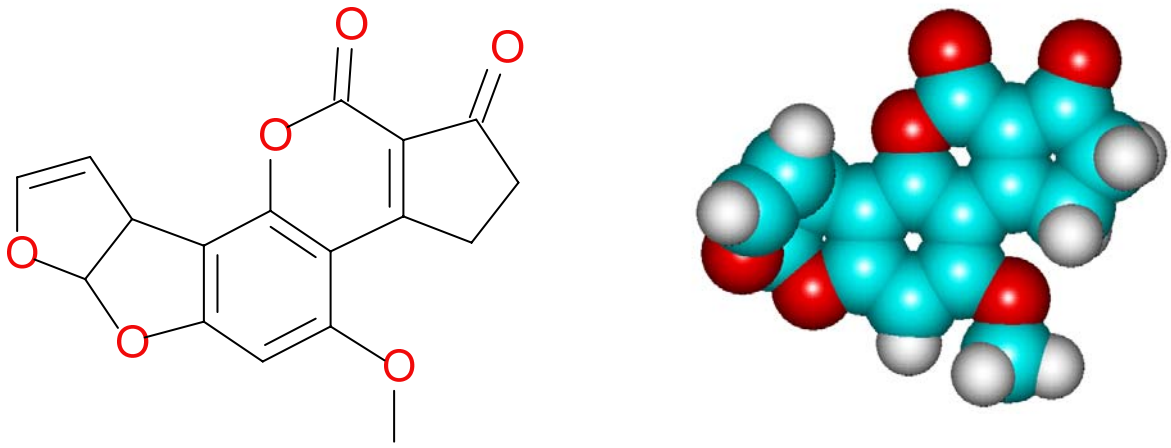

Figure 11. 2D and 3D structures of Aflatoxin B_1.

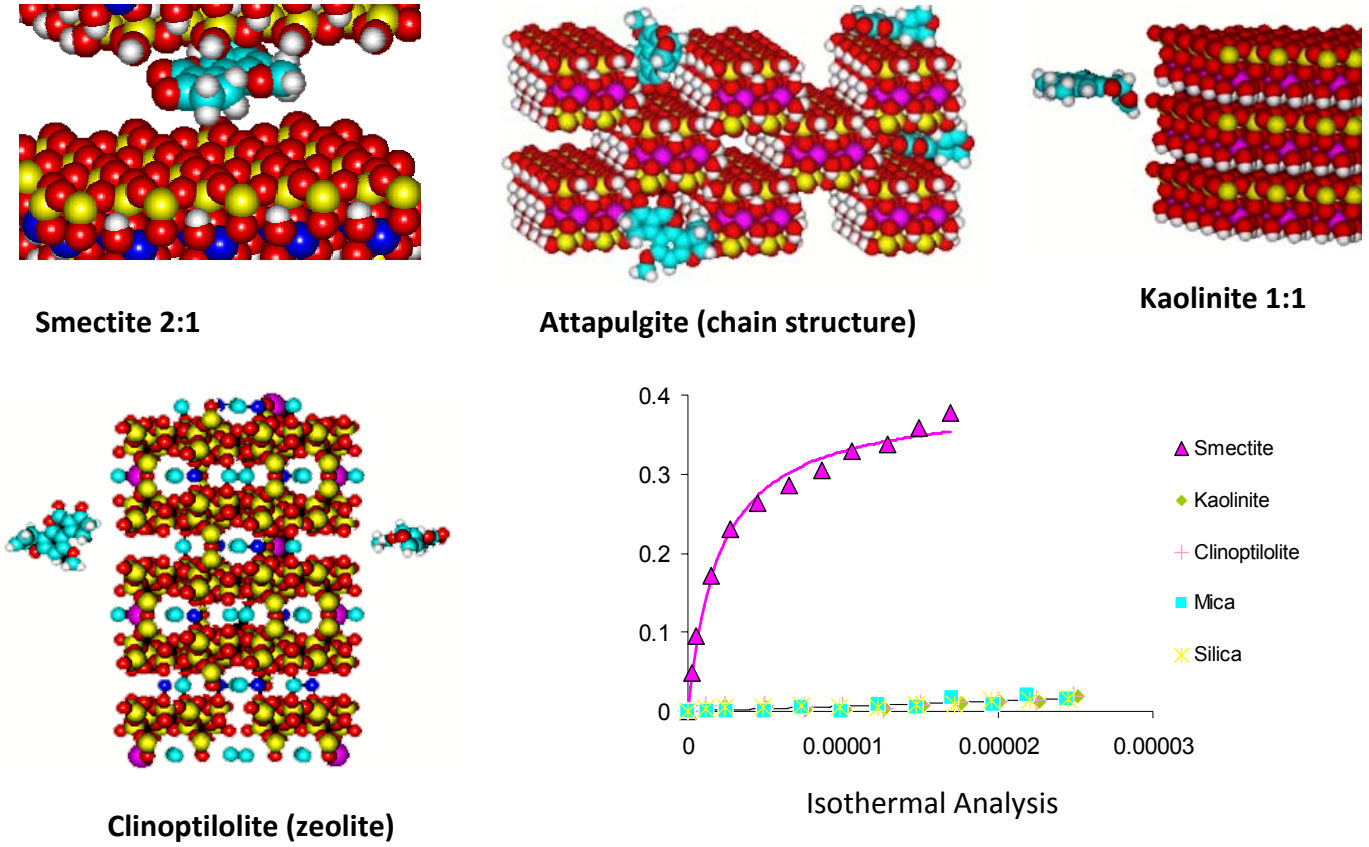

Figure 12. Structure activity relationships of potential aflatoxin binding soil minerals. 3D models show the steric hinderance of attapulgite, kaolinite and clinoptilolite versus that of smectite for binding of aflatoxin. Isothermal analysis verifies that smectite is a high capacity sorbent for aflatoxin.

in multiple animal models and even humans (*70-80*). The mechanism of binding appears to be an electron donor acceptor (EDA) mechanism. This is due to the negative charge on the surface the layers of NS that attract the areas of the aflatoxin molecule that have electron deficiencies, specifically the carbons that make up the dicarbonyl system (Figure 11). AfB1 is planar and its spatial orientation has been found to be important for binding of AfB1 and other congeners of aflatoxin (*67*). Experiments on heat-collapsed NS have confirmed the importance of an intact interlayer on the binding of aflatoxin (*69*) (Figure 12). The success of NS as an enterosorbent serves as a model for how a selective sorbent can be effectively implemented to impact the health of those exposed chronically to a toxic agent.

Dietary Iron Requirements and Absorption

Iron is an essential dietary nutrient to animals including humans. Iron is an essential component of proteins involved in oxygen transport, and it is also essential for the regulation of cell growth and differentiation (*81, 82*). Iron deficiencies limit oxygen delivery to cells, resulting in fatigue and decreased immunity. Over half of the iron in the body is bound to hemoglobin, the protein in red blood cells that carries oxygen to tissues. Smaller amounts can be found in myoglobin, a protein important for supplying oxygen to muscle, and in enzymes assisting biochemical reactions. Iron is also found in storage and transport proteins in the blood. Iron stores are regulated by iron absorption (*81, 83*).

There are two forms of dietary iron, heme and nonheme. Heme iron is derived from hemoglobin, and is found in foods from animal origins that

contained hemoglobin such as red meats, fish, and poultry (Table 4). Iron from plants or other sources is called nonheme iron, and this is the form most often added as a dietary supplement (*84*). Examples of nonheme iron sources are summarized in Table 5.

Iron absorption means the portion of the iron from the diet that the body actually uses. In healthy adults about 10 to 15% of dietary iron is actually absorbed, however this can vary depending on several factors (*81*). Stored iron levels have the largest impact on iron absorption, as more iron is absorbed when stores are low (*81, 85*). Iron absorption can also vary by type of iron consumed. Absorption of heme iron ranges from 15 to 35%, and is not overly affected by diet (*86*). Nonheme iron, however, can vary from 2 to 20% (*87*). Vitamin C containing foods or supplements tend to increase iron absorption, while tannins, calcium, polyphenols, and phytates (from legumes and whole grains) can decrease absorption of nonheme iron (*81, 88, 89*). This makes it very important to consume foods that enhance nonheme iron when iron intake is low, when iron losses are high such as during menstruation, when iron requirements are high such as in pregnancy, or when the sole source of iron is from vegetarian sources. Recommended daily allowances are summarized in Table 6 and tolerable daily intakes are summarized in Table 7.

The World Health Organization has stated that as many as 80% of the world's population is iron deficient, while up to 30% may suffer from iron deficiency anemia (*90, 91*). Iron deficiency develops slowly as iron intake does

Table 4. Heme iron found in common foods (92).

Food	Milligrams per serving	% DV
Chicken liver, cooked 3.5 oz.	12.8	70
Oysters, breaded and fried, 6 pieces	4.5	25
Beef, chuck, lean only, braised 3 oz.	3.2	20
Clams, breaded, fried, 3/4 cup	3	15
Beef, tenderloin, roasted, 3 oz.	3	15
Turkey, dark meat, roasted, 3.5 oz	2.3	10
Beef, eye of round, roasted, 3 oz.	2.2	10
Turkey, light meat, roasted, 3.5 oz.	1.6	8
Chicken, leg, meat only, roasted 3.5 oz.	1.3	6
Tuna, fresh, bluefin, cooked, dry heat 3 oz.	1.1	6
Chicken, breast, roasted, 3 oz.	1.1	6
Halibut, cooked, dry heat, 3 oz.	0.9	6
Crab, blue crab, cooked moist heat, 3 oz.	0.8	4
Pork, loin, broiled, 3 oz.	0.8	4
Tuna, white, canned in water, 3 oz.	0.8	4
Shrimp, mixed species, cooked moist heat, 4 large	0.7	4

Table 5. Nonheme iron from common foods (92).

Food	Milligrams per serving	% DV
Ready-to-eat cereal, 100% iron fortified, 3/4 cup	18	100
Oatmeal, instant, fortified, prepared with water, 1 cup	10	60
Soybeans, mature, boiled, 1 cup	8.8	50
Lintels, boiled, 1 cup	6.6	35
Beans, kidney, mature, boiled, 1 cup	5.2	25
Beans, lima, large, mature, boiled, 1 cup	4.5	25
Beans, navy, mature, boiled, 1 cup	4.5	25
Ready-to-eat cereal, 25% iron fortified, 3/4 cup	4.5	25
Beans, black, mature, boiled, 1 cup	3.6	20
Beans, pinto, mature, boiled, 1 cup	3.6	20
Molasses, blackstrap, 1 tablespoon	3.5	20
Tofu, raw, firm, 1/2 cup	3.4	20
Spinach, boiled, drained, 1/2 cup	3.2	20
Spinach, canned, drained solids, 1/2 cup	2.5	10
Black-eyed (cowpeas), boiled, 1 cup	1.8	10
Spinach, frozen, chopped, boiled 1/2 cup	1.9	10
Grits, white, enriched, quick, prepared with water, 1 cup	1.5	8
Raisins, seadless, packed, 1/2 cup	1.5	8
Whole wheat bread, 1 slice	0.9	6
White bread, enriched, 1 slice	0.9	6

Table 6. Recommended daily allowances for infants, children, and adults (81).

Age	Males (mg/day)	Females (mg/day)	Pregnancy (mg/day)	Lactation (mg/day)
7 to 12 months	11	11	NA	NA
1 to 3 years	7	7	NA	NA
4 to 8 years	10	10	NA	NA
9 to 13 years	8	8	NA	NA
14 to 18 years	11	15	27	10
19 to 50 years	8	18	27	9
51+ years	8	8	NA	NA

Table 7. Tolerable daily intakes for infants, children, and adults (81).

Age	Males (mg/day)	Females (mg/day)	Pregnancy (mg/day)	Lactation (mg/day)
7 to 12 months	40	40	NA	NA
1 to 13 years	40	40	NA	NA
14 to 18 years	45	45	45	45
19+ years	45	45	45	45

not meet dietary needs. The storages of iron begin to be depleted, which may not affect hemoglobin level, a marker of iron status. Iron deficiency anemia is an advanced state of iron depletion. Symptoms include feeling tired or weak, decreased work or school performance, slow cognitive or social development during childhood, difficulty maintaining body temperature, decreased immune function, and glossitis (inflamed tongue) (*81, 93, 94, 95*). People deemed to be at risk include those with inadequate intake of iron, women of childbearing age, pregnant women, preterm or low birth weight infants, older infants and toddlers, teenage girls, and women with high menstrual losses, all because they have the most need for dietary iron (*81, 85*). Since vitamin A helps mobilize iron from storage sites, vitamin A deficiency can contribute to iron deficiency as well. Though uncommon in the US, this problem can be common in developing countries where vitamin A deficiency is more widespread (*96, 97*).

There is potential for iron toxicity because very little is excreted. Iron can accumulate in tissues and organs even if stores are full (*81*). This can be exacerbated in individuals who have the disease hemachromatosis, where individuals store more iron that normal (*98*). For this reason, any treatment using iron oxides as an enterosorbent would need to be closely monitored to ensure safety on an individual basis.

Reseach Goals and Objectives

The overall goal of this research was to find and characterize an enterosorbent for As to reduce effects seen from chronic ingestion of As in water. Previous studies on the bioavailability of As from soil have demonstrated that As

may be tightly bound to mineral oxides from soil and the complex may remain bound throughout the gastrointestinal tract, thus reducing As bioavailability and toxicity from water. A similar strategy of enterosorption therapy has been shown to be highly effective for aflatoxin using montmorillonite clays.

The first objective of this project was to screen diverse sorbents from various classes of inorganic, organic and biological materials for their ability to bind As as arsenite and arsenate. Although high surface area oxide minerals such as ferrihydrite were expected to be the best sorbents for As, many previous studies have not directly compared the binding of a wide variety of sorbents in the same experiments. These experiments served to directly compare the various sorbents. Since both As species are commonly found in groundwater due to slow redox reactions, it was important was to find a sorbent that bound both arsenite and arsenate. Because a non-selective sorbent may bind important nutrients, including vitamins, ferrihydrite and other targeted soil minerals were tested for the sorption of vitamin A (VA) and riboflavin (RF), model fat-soluble, and water soluble vitamins.

After selecting an optimal sorbent for As (i.e., ferrihydrite), it was further characterized for its ability to sorb As using isothermal analysis to delineate binding capacity. It was then evaluated using an in vitro gastrointestinal model which simulated conditions likely to be encountered during ingestion. Next the sorbent was tested for safety and efficacy using the adult Hydra bioassay. These studies were designed to verify ferrihydrite's capacity and ability to bind As at physiological pH.

Finally, ferrihydrite was tested in a rat model. Rats were dosed via gavage with either sorbent plus As or As only. Measurements of total urinary As served to determine if the sorbent was able to reduce biomarkers of As exposure. Short term safety of enterosorbent therapy using ferrihydrite was evaluated in rats given 0.5% sorbent w/w in their diet for a period of two weeks. At the end of two weeks, organs were observed for gross lesions, and blood was drawn for measurement of serum biochemistry and serum Fe. These experiments served to delineate short term safety and proof of concept for use of ferrihydrite as an enterosorbent for As.

CHAPTER II

DEVELOPMENT AND SCREENING OF COMPOSITE AND SOIL MINERAL MATERIALS FOR THE SORPTION OF ARSENIC FROM WATER*

Introduction

Arsenic is a toxic metalloid that is found in groundwater from natural, industrial and agricultural processes. Arsenic in drinking water is a problem throughout the world, highlighted by exposures in Bangladesh and Taiwan. Adverse health effects from As exposure include various cancers, skin lesions, neurological effects, hypertension and cardiovascular disease, pulmonary disease, peripheral vascular disease, and increased incidence of diabetes mellitus (*2*). Inorganic arsenic is the most common and important form of arsenic found in groundwater with arsenite and arsenate being the main forms of inorganic As. Arsenite is predominant under reducing conditions which are frequently found in groundwater, whereas arsenate is predominant under oxidizing conditions. Slow redox conversion of the two species leads to both forms being present in either environment (*55*), and thus any treatment or filter technology intended for the removal of arsenic from water must be adept at removing both arsenic species.

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* Portions of this chapter are reproduced in part with permission from Taylor JF, Robinson A, Johnson N, Marroquin-Cardona A, Brattin B, Taylor R, Phillips TD. In vitro evaluation of ferrihydrite as an enterosorbent for arsenic from contaminated drinking water. *Environ Sci Technol*. **2009** Jul 15;43(14):5501-6.

Previous research identified iron oxides and oxy-hydroxides as effective sorbents for removing inorganic arsenic from water (*54, 56*). Ferrihydrite has been shown to be considerably more effective than crystalline iron oxides as well as phyllosilicate minerals such as illite, montmorillonite and kaolinite in removing inorganic arsenic from water (*54*). The As binding reaction with ferrihydrite is pH dependent with arsenate being adsorbed best at lower pHs and arsenite at higher pHs. The two species are equally adsorbed at approximately pH 6-7.5. At higher pHs, arsenate was found to steadily decrease in amount adsorbed, while arsenite remained highly bound at increasing pHs up to about 10 (*56*).

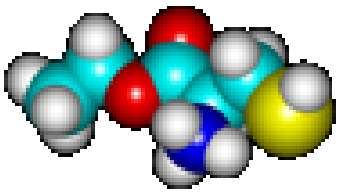
The goal of this study was to screen different sorbents for their effectiveness at removing arsenate and arsenite from water and to compare their effectiveness for ferrihydrite. Emphasis was on selection of sorbents that could potentially be fed in the diet as an enterosorbent for As. We tested two montmorillonite minerals that have been shown to be safe in animal studies (*99*), an organoclay used to sorb PAH's and pentachlorophenols (*100*), organoclays amended with sulfhydral and disulfide groups for sorption of heavy metals (*101*), crystalline iron oxides and oxy-hydroxides (which have a comparably low surface area versus ferrihydrite), attapulgite, muscovite (a 2:1 mineral with a low surface area), halloysite (a 1:1 mineral), synthesized 2-line ferrihydrite, and an industrially produced ferrihydrite (IPF) for their ability to sorb arsenate and arsenite from water. Because sorbents that are nonselective may interact with nutrients and pose significant hidden risks (*102*), we evaluated selected sorbents for their ability to sorb vitamin A (VA) and riboflavin (RF).

Chemical Reagents and Sorbents

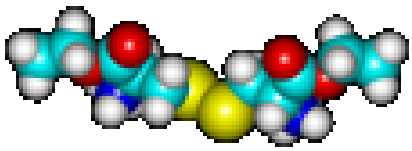
All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) and were at least ACS reagent grade. Goethite was obtained from Sigma, while kaolinite, muscovite, clinoptilolite, attapulgite, halloysite, and SWy-2 were obtained from the Clay Mineral Repository (Purdue University, Indiana). Low pH montmorillonite (LPHM), IPF (industrially produced ferrihydrite), NovaSil (NS) and activated carbon (810-6, KB-B) were obtained from BASF corporation (Jackson, MS). Goethite, 2-line ferrihydrite, and magnetite were prepared according to Schwertmann and Cornell, 2000 (*50*). All iron oxides were air-dried in a fume hood and ground and sieved to <100 *μ*m. Iron oxides were verified by X-ray diffraction, and their surface areas were measured after degassing at 110 °C for 2 h (*56*) using a multipoint BET analysis with N_2 adsorption on a Micromeritics ASAP 2020. CP-LPHM was prepared according to Lemke et al. (*104*). SWy-2 was exchanged at 100% of its CEC with L-cysteine ethyl ester, L-cysteine dimethyl ester, and thiamine to make SW-CYSTE, SW-CYSTI, and SW-THIAM, respectively, using previously established methods (*101*) (Figure 13). Collapsed NS and SWy-2 were prepared by heating the clays to 200°C for 30 min. and then heating the sample at 800°C for 1 hr.

Arsenic Screening Procedure

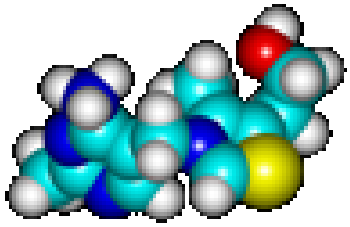
Stock solutions of 9.375 and 37.5 ppm As(V) and As(III), respectively, were prepared in water from sodium arsenate and sodium metarsenite, respectively. The final pH of each solution was adjusted to 7. Into 15mL polypropylene tubes, 4 mL of the stock solution of each specie of arsenic was



L-cysteine ethyl ester (CYSTE)



L-cystine dimethyl ester (CYSTE)



Thiamine (THIAM)

Figure 13. Three dimensional representations of sulfur containing cations exchanged onto the surfaces of SWy-2.

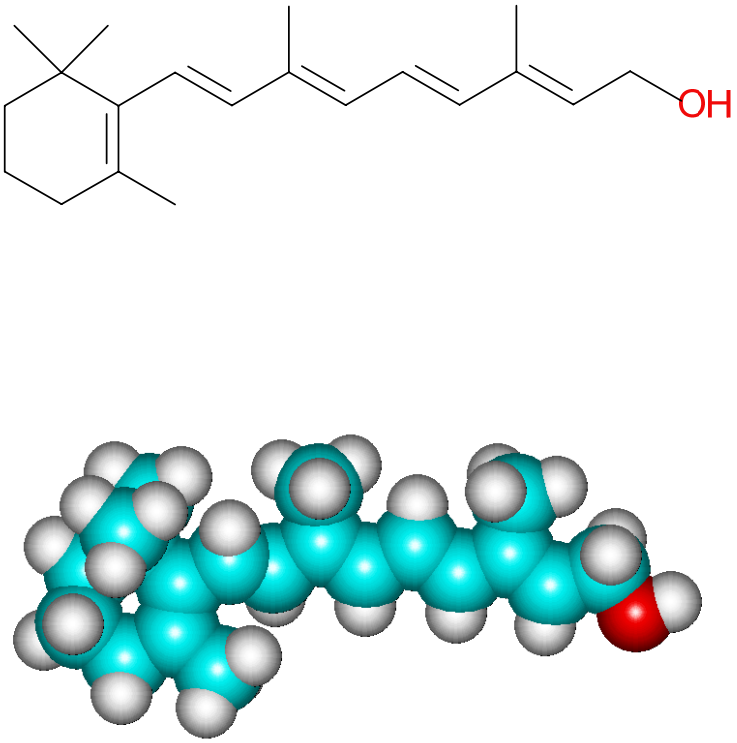


Figure 14. 2D and 3D images of vitamin A as retinol.

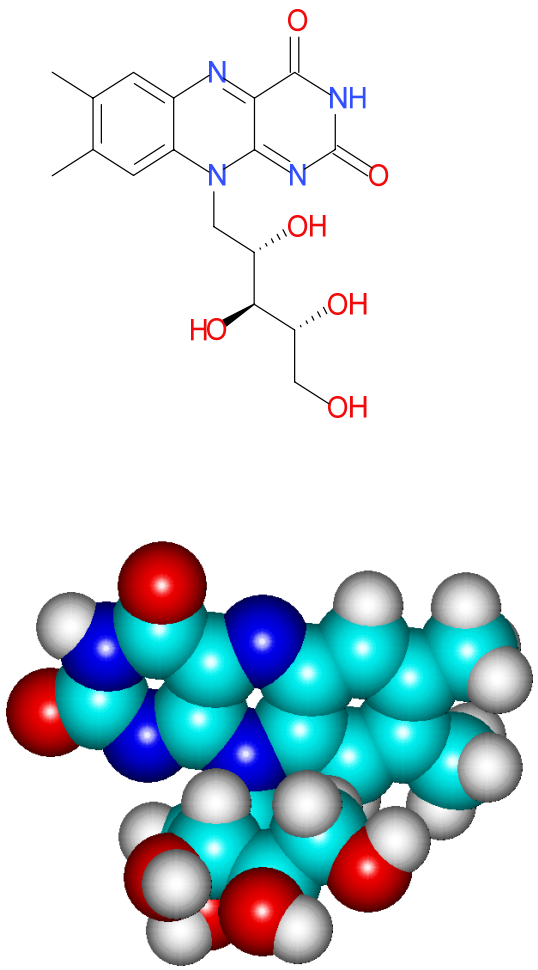


Figure 15. 2D and 3D images of riboflavin.

added along with 1 mL of a 5 mg/mL slurry of sorbent for a final concentration of 1 mg/ mL sorbent and 7.5 ppm As(V) or 30 ppm As(III). The final concentrations were based on previous work showing that 1 mg/mL ferrihydrite in a total of 5 mL solution bound 98% of 7.5 ppm As(V) in water (*104*) and from Raven et al. who found the adsorption maxima of As(III) on ferrihydrite to be nearly 4 times higher than As(V) at higher pH values (*56*). The tubes were then agitated at 1000 rpm on a shaker at 25°C for 4 h. They were then centrifuged at 2500 rpm for 20 minutes, and 2.5 mL of the supernatant was saved for arsenic analysis. Each test was done in triplicate. All As analysis was by inductively coupled plasma-optical emission spectroscopy (ICP-OES).

Isothermal Adsorption of Vitamin A and Riboflavin

Stock solutions of VA (Figure 14) were prepared by dissolving the pure crystals in 100% ethanol. RF (Figure 15) stock solutions were prepared by dissolving pure crystals in purified water. Appropriate volumes of VA or RF stock solutions were then added to water to yield 10 µg/mL solutions of the individual vitamins. Test samples for each vitamin were made in triplicate and contained increasing concentrations of VA or RF ranging from 0.5 to 10 µg/mL with 20 µg/mL sorbent for a total volume of 5 mL. These were agitated at 1,000 rpm for 2 h at 25°C in the dark and then centrifuged at 2,000 rpm for 20 min at 25°C. The concentration of vitamin remaining in the solution was determined from the resulting supernatents by UV-visible spectrophotometry at 292 nm and 445 nm for VA and RF respectively.

Data Calculations and Curve Fitting

The UV-visible absorption data were used to calculate the amount of VA or RF left in solution and the amount sorbed for each treatment. Isothermal data were transferred to Table Curve 2D software (version 3, Genesis Technologies, Inc. Austin, TX) and fit with the Langmuir equation to estimate capacity values (Q_{max}) and the distribution constants (K_d) (Figure 16).

Enthalpy Calculations

To estimate the enthalpy of sorption of RF to SWy-2 and NS, experiments were repeated with selected sorbents at 15° and 37°C. The enthalpy of sorption was calculated from the experimental data using the van't Hoff equation (Figure 16).

Chitosan Gel Beads

Chitosan gel beads were prepared by dissolving 1.0 g of chitosan in 100 mL of 1 M acetic acid and dropping the solution through a glass pipette tip into 1 M NaOH with the tip placed approximately 6-7 cm over the NaOH solution. The beads (2-3 mm in diameter) were then aged in the 1 M NaOH for 24 hours and dialyzed until the solution was at pH 7. The beads were stored in pure H_2O (18.2 MΩ.cm) until ready for use. Ferrihydrite amended beads were prepared by adding 1.0 g of the iron oxide with 1.0 g of chitosan and proceeding as previously

Langmuir Equation:

$$q = Q_{max}*(K_dC_w)/(1+K_dC_w)$$

Q_{max} = maximum amount of sorbent bound

K_d = distribution constant

C_w = equilibrium concentration of ligand in solution

q= concentration of ligand bound

van't Hoff Equation:

$$\Delta H_{ads} = \frac{-R \ln (K_{d2}/K_{d1})}{(1-T_2)-(1/T_1)}$$

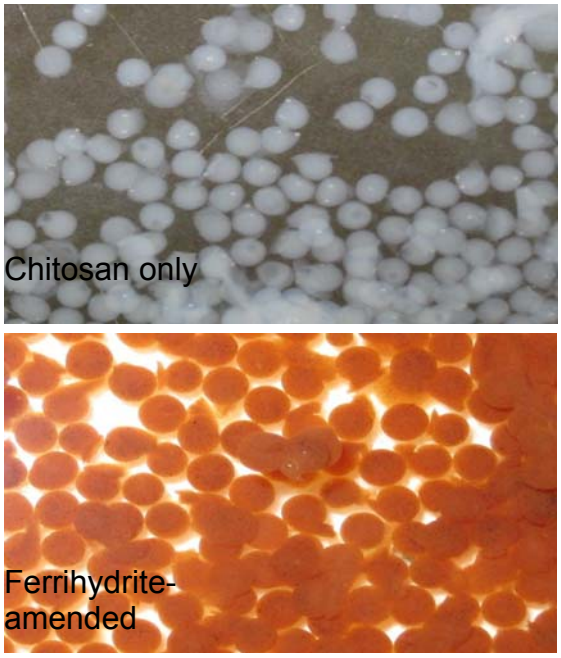
R = Gas Constant

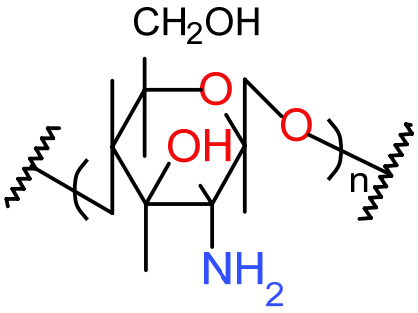
K_d = distribution constant

T = Temperature

ΔH_{ads} = Enthalpy of adsorption

Figure 16. Langmuir and van't Hoff equations.





Chitosan

Figure 17. Pictures of chitosan gel beads (left). Beads measured 2-3 mm in diameter. On the right is the chemical structure of chitosan. The proposed mechanism of As binding (and other heavy metals) is by attraction to the amine group.

described (Figure 17). To assess the ability of chitosan gel beads to sorb As from water, 1.0 g of beads (with and without ferrihydrite) were added to 100 mL of water containing 25 ppm arsenate or arsenite at pH 7. The reaction vessels were placed on an orbital shaker and shaken at 1000 rpm for 24 hours at 25°C. Triplicate 5 mL aliquots were then taken for As analysis via ICP-OES. Chitosan Gel beads have previously been shown to be effective at removing As from water (*105*), and they would also be a means of coating ferrihydrite for protection from the acidic conditions of the stomach if needed for its application as an enterosorbent.

Arsenic Analysis by ICP-OES

Sample extracts were diluted 1:1 with a solution containing deionized water, 2% nitric acid (v/v), and 10% hydrochloric acid (v/v) in order to match the sample matrix to the standards used to calibrate the instrument. Samples were then analyzed for As using a Spectro CirOS axial ICP-OES (Spectro AI, Fitchburg, MA) employing a modified version of USEPA Method 200.7 (*106*). The diluent was analyzed as an independent sample, which showed no traces of As at the levels measured in the samples. The instrument was calibrated using a series of standards (As 100 ppm) at approximately twice the concentration in solution observed in the highest test sample for As. The calibration was verified against independent standards and the calibration blank before the analytical run, approximately every ten samples during the run, and at the end of the analytical run. Ytterbium was employed as an internal standard.

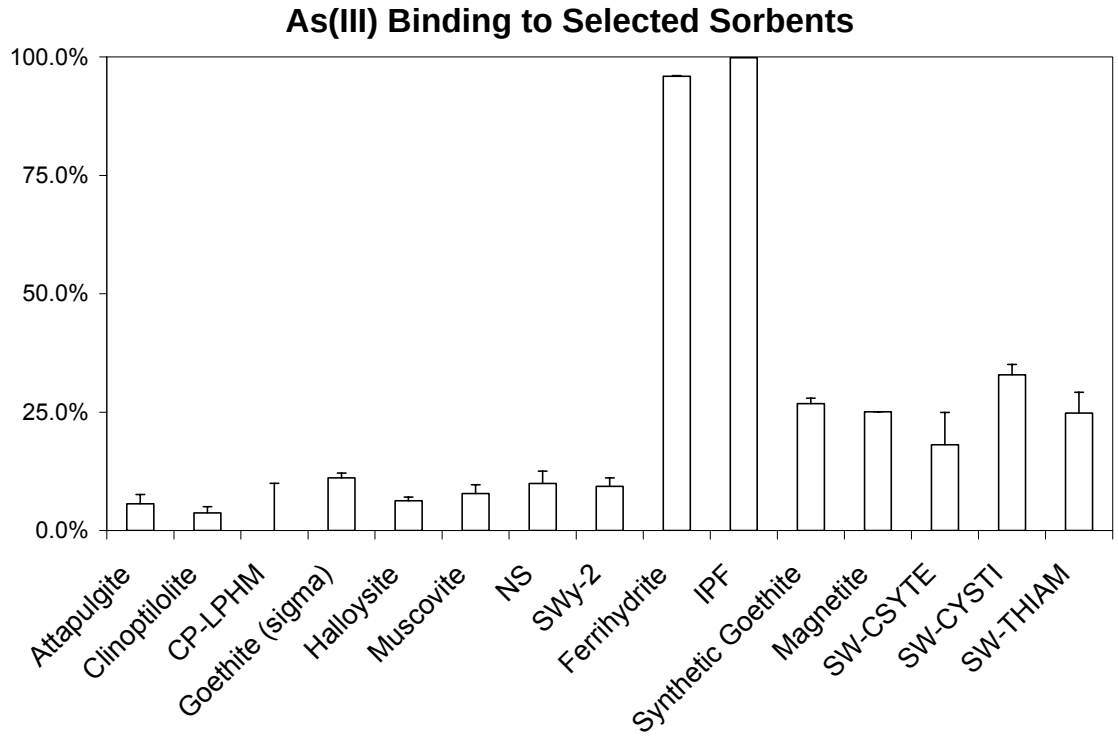


Figure 18. As(III) binding to selected sorbents. Initial As(III) concentration was 30 ppm and sorbent concentration was 1 mg/mL. Ferrihydrite and IPF bound 95.9% and 99.8%, respectively, of the As(III) in solution while the exchanging of SWy-2 with S containing groups increased the binding of As(III) from 9.3% to 18.1%, 32.9%, and 24.8% for SW-CYSTE, SW-CYSTI, and SW-THIAM, respectively.

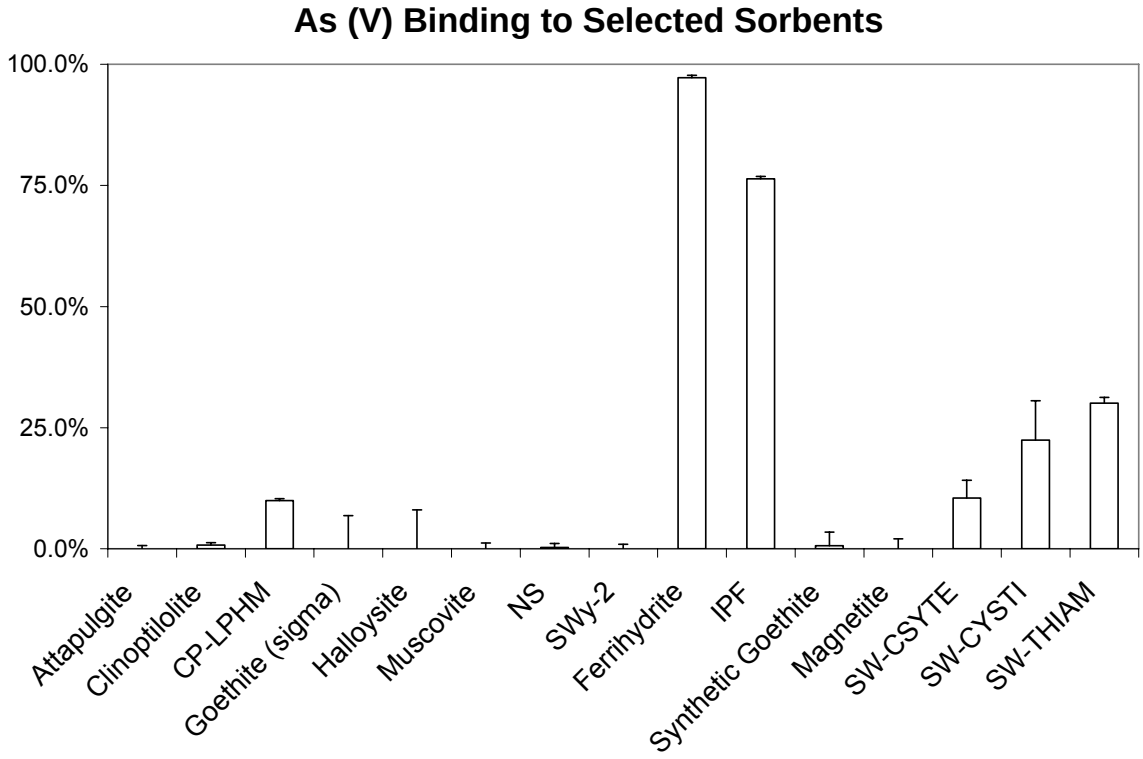


Figure 19. As(V) binding to selected sorbents. Initial As(V) concentration was 5 ppm, and sorbent concentration was 1 mg/mL. Ferrihydrite and IPF bound 97.2% and 76.4%, respectively of the As(V) in solution. The exchanging of SWy-2 lead to increases in binding from 0.0% for SWy-2 to 10.5%, 22.45%, and 30.1% for SW-CYSTE, SW-CYSTI, and SW-THIAM, respectively.

Results and Discussion

Ferrihydrite proved to be the most effective sorbent in the screening experiments, binding 95.9% and 97.2% of the As(III) and As(V) in solution (Figures 18, 19). IPF also proved to be highly effective removing 99.8% of As(III) and 76.4% of As(V). Attapulgite and clinoptilolite both have very large surface areas but the pore sizes in attapulgite (palygorskite) are too small for the sorption of certain molecules while clinoptilolite's channel diameter is too small to allow all but the smallest molecules to enter (*52*). Neither is an effective sorbent for As(III) or As(V) when compared to the iron oxides and ferrihydrite. The two montmorillonite minerals, NovaSil and SWy-2, sorbed virtually none of the As(V) but bound 9.9% and 9.3%, respectively of the As(III). The exchanging of SWy-2 with sulfur containing organic cations greatly increased the sorption of both As species to. SW-CYSTE, SW-CYSTI, and SW-THIAM suggesting that they could be effective at removing As from water. They have previously been shown to remove heavy metals from water including Hg (*101*). However, this is the first report of these clay-based composites working for As. The exchanging of low pH montmorillonite (LPHM) with cetyl pyridinium (CP) increased the binding of As(V) when compared to the other montmorillonites. Since we exceeded a 1:1 stoichiometry for exchange, this effect could be due to an excess of the organic cations on the clay surface which have been shown to result in a positively charged clay. The positively charged clay may then attract As(V), which would be in the $H_2AsO_4^-$ or $HAsO_4^{2-}$ state at neutral pH. As(III) exists as H_3AsO_3 at neutral pH which could explain CP-LPHM's lack of affinity for As(III). Halloysite

was chosen to represent the 1:1 class of phyollosilicate clays due to its hydrated interlayer which allows for more of its reactive Al-OH bonds to be available for As sorption. Kaolinite is more closely packed in its formation with the layers being hydrogen bonded and thus unavailable for As sorption (*52*). Halloysite, was found to be far less effective than ferrihydrite, binding essentially no As(V) and only 6.3% of the As(III) in solution. It was clear from these screening experiments that ferrihydrite had a much higher capacity for the sorption of arsenite and arsenate than any of the other sorbents tested. The clays exchanged with S containing groups showed moderate binding compared to ferrihydrite suggesting they may be effective for applications needing a multi-purpose sorbent of heavy metals and arsenic. Although not tested by the original authors (*101*), these clays could also potentially sorb cadmium, making for an even more versatile sorbent. One concern about organoclays as entersorbents is the potential for the exchanged cation to become bioavailable in the stomach or intestines. With these clays, that cation is either a vitamin or an amino acid, which could contribute to the safety and acceptance of these organoclays as enterosorbents for heavy metals and maybe arsenic. Because we were specifically looking for an arsenic enterosorbent, we chose to continue in vitro and in vivo tests with ferrihydrite, as it showed the most potential as a potential As enterosorbent.

To test possible nutrient interactions with ferrihydrite we conducted isotherms for VA and RF for a variety of sorbents (Figures 20, 21). Activated carbon was chosen as a positive control for the binding of both VA and

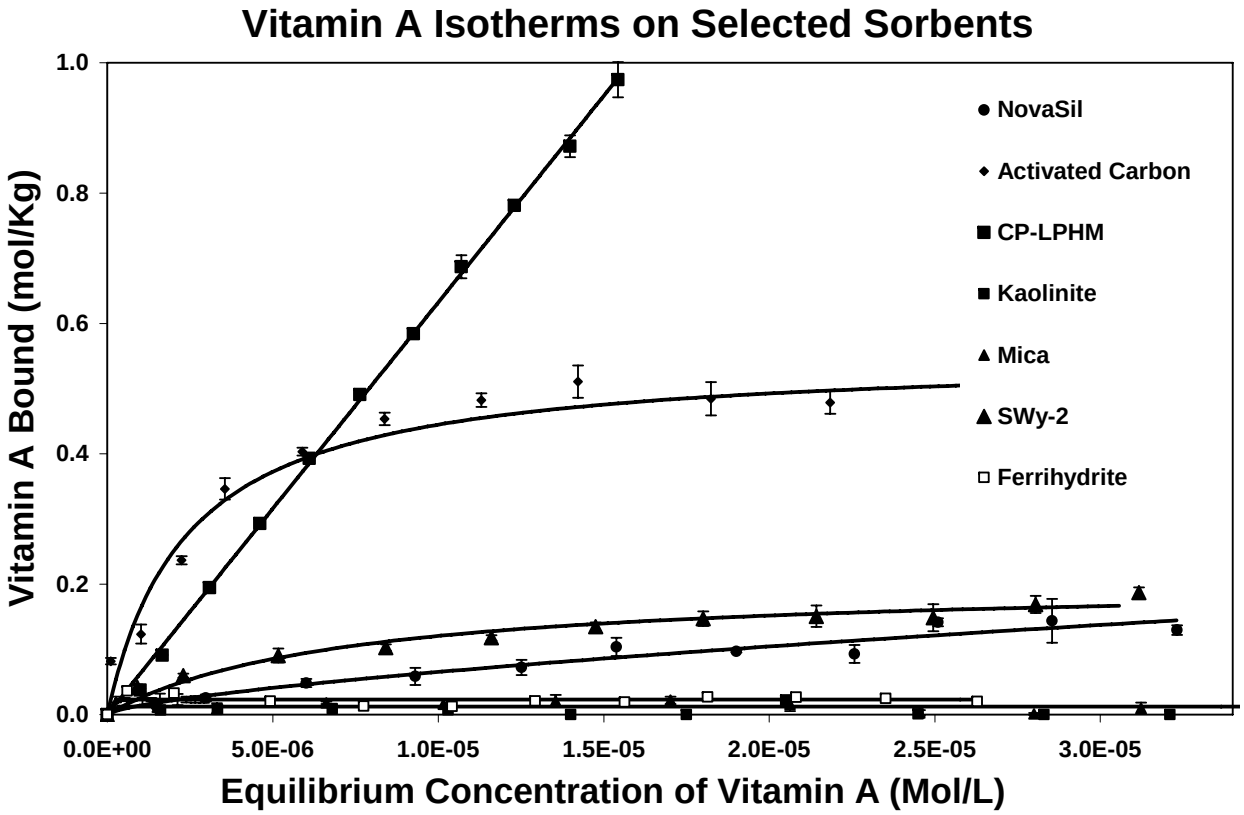


Figure 20. Vitamin A isotherms performed on selected sorbents. CP-LPHM and activated carbon both sorbed VA at high capacity as expected, with activated carbon being a non-selective sorbent and CP-LPHM being an organophilic clay. Binding of VA to the remainder of the sorbents tested was lower and validates in vivo data for montmorillonites.

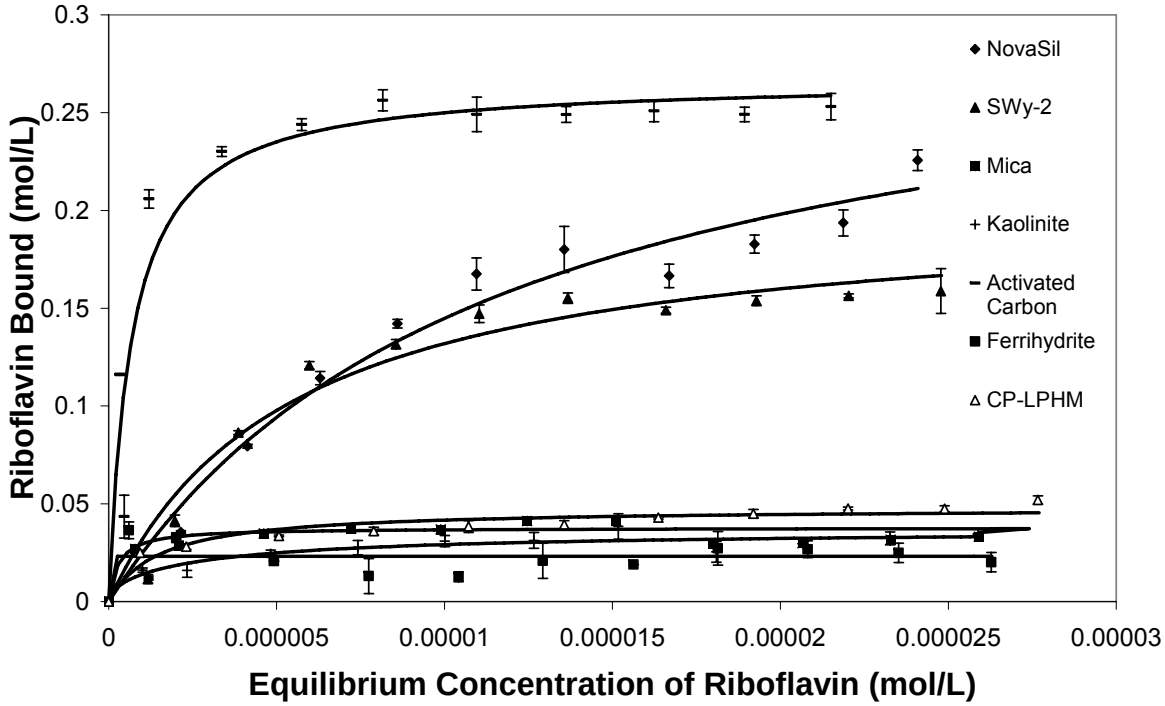


Figure 21. Riboflavin isotherms performed on selected sorbents. Activated carbon and the two montmorillonites tested, NS and SWy-2 showed significant binding of riboflavin. Other sorbents tested, including ferrihydrite, sorbed only a small amount of riboflavin from solution.

RF as its porous, high surface area allows for the sorption of diverse compounds. For this reason, it would be undesirable as an enterosorbent due to its lack of selectivity. Isotherms of RF and VA on activated carbon confirm this lack of selectivity as it sorbs both species at high capacity as exhibited by Q_{max} values of 0.267 mol/kg and 0.552 mol/kg, respectively. CP-LPHM is an organophilic clay which could interact with other organophilic compounds such as VA as demonstrated by its Q_{max} of 4.000 mol/kg for VA. The binding curve for VA to CP-LPHM is a C type isotherm curve indicating a likely partitioning of VA into the organophilic interalayer of the organoclay. Other soil minerals and ferrihydrite showed far less affinity for VA, and their interaction with VA was unlikely in vivo based on the results of the isotherms. NS has been shown to rescue VA levels in the liver of broiler chickens exposed to aflatoxin (*67*). VA plays an important role in immune function and overall health, which highlights the importance of using a selective sorbent for long term enterosorption therapy.

Besides activated carbon, the montmorillonites, SWy-2 and NS, were also found to sorb RF, exhibited by Q_{max} values of 0.193 and 0.313 mol/kg, respectively. Riboflavin contains electron withdrawing moities such as N's and O's in its structure, and is also planar, both of which have been found to play a significant role in aflatoxin sorption to montmorillonites. The importance of an intact interlayer for the binding of RF was verified by low binding by mica, which has a similar 2:1 structure to montmorillonite but with a potassium fixed interlayer (*52*). Experiments on collapsed SWy-2 and NS also confirmed this conclusion

(Figures 22, 23). RF and other flavins have previously been shown to sorb on the surfaces of smectite clays, but this interaction is weak (*107, 108*). High enthalpy of sorption (ΔH>-20 kJ/mol) has been hypothesized to be a reason that aflatoxin is tightly sorbed to smectite clay with resulting effectiveness in vivo (*67*). Enthalpy of sorption ranged from -5.03 kJ/mol to 8.57 kJ/mol with a predicted value of 0.3 kJ/mol for sorption of RF to SWy-2, while NS had a range from -4.44 kJ/mol to 19.31 kJ/mol with a predicted value of 1.1 kJ/mol (Figures 24, 25). In studies in poultry, Chung and Baker showed that NS did not affect RF utilization, and these results agree with their findings (*109*). Although the goal was to find a enterosorbent for As, these results are important as smectite clays, and the other soil minerals tested, could be constituents of any naturally mined source of ferrihydrite that would be used as an enterosorbent for As. Additionally, ferrihydrite was not shown to sorb either VA or RF which would be important for potential nutrient interactions.

Chitosan gel beads have previously been shown to be effective for As(III) and As(V) adsorption (*105*). Ferrihydrite added 1/1 with chitosan during synthesis increased the sorption of As(III) from 26.7% to 60.8% and from 23.7% to 33.7 for As(V) compared to chitosan only beads (Figure 26). Inclusion of ferrihydrite is an easy process and could enhance chitosan gel beads' effectiveness at removing As from water when used in applications such as clean up columns. For the purpose of using ferrihydrite as an enterorsorbent, encapsulation with chitosan beads could serve as an enteric coating should acid dissolution of ferrihydrite pose a problem with its action. These data illustrate the

versatility of a chitosan gel bead composite sorption system that could be modified with various sorbents for toxin removal.

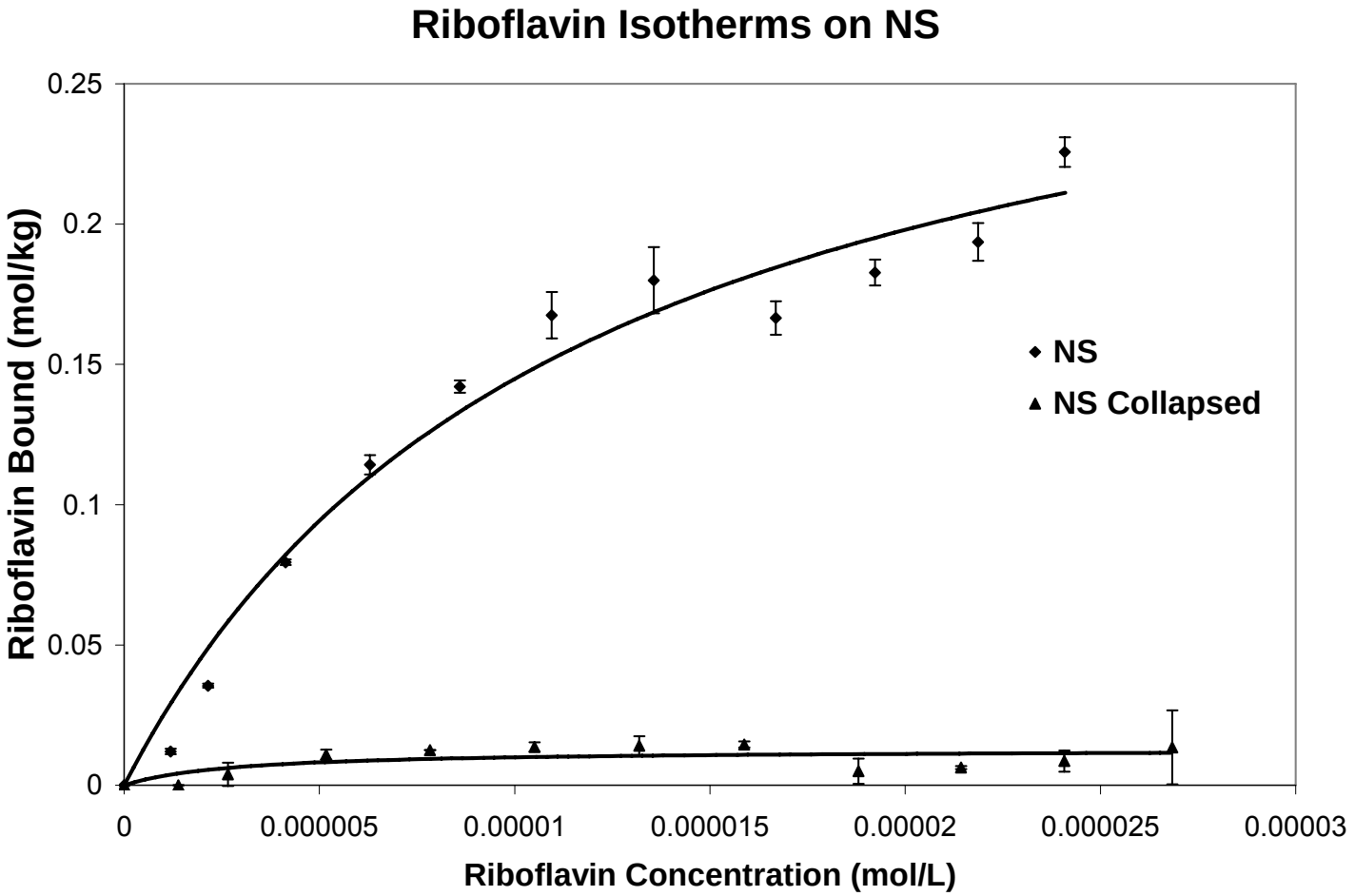


Figure 22. Riboflavin isotherms on NS and heat collapsed NS. These experiments clearly showed the importance of an intact interlayer for the binding of RF by NS. Error Bars represent ± 1 standard deviation.

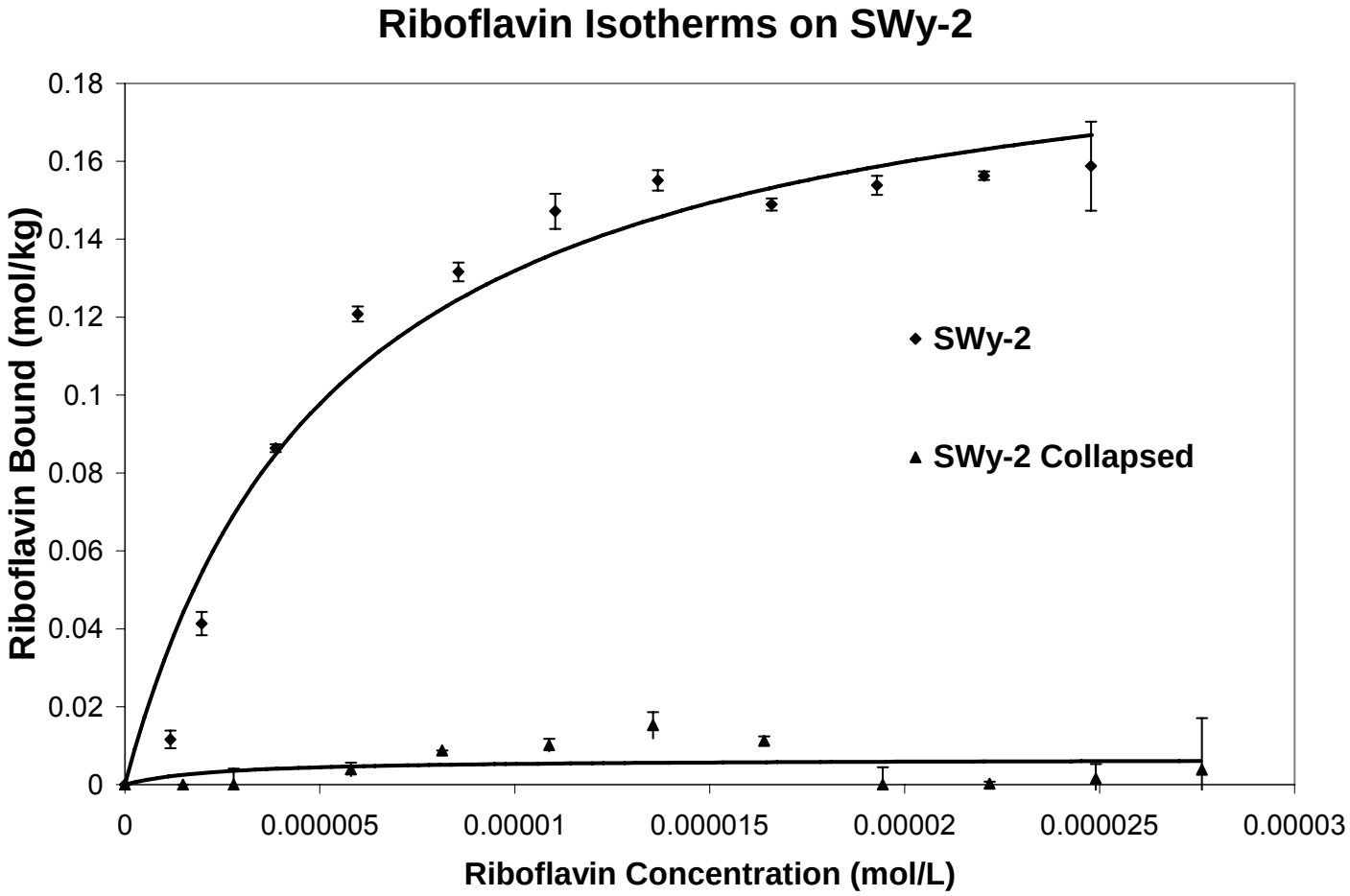


Figure 23. Riboflavin isotherms on SWy-2 and heat collapsed SWy-2. These experiments clearly showed the importance of an intact interlayer for the binding of RF by SWy-2. Error bars represent ± 1 standard deviation.

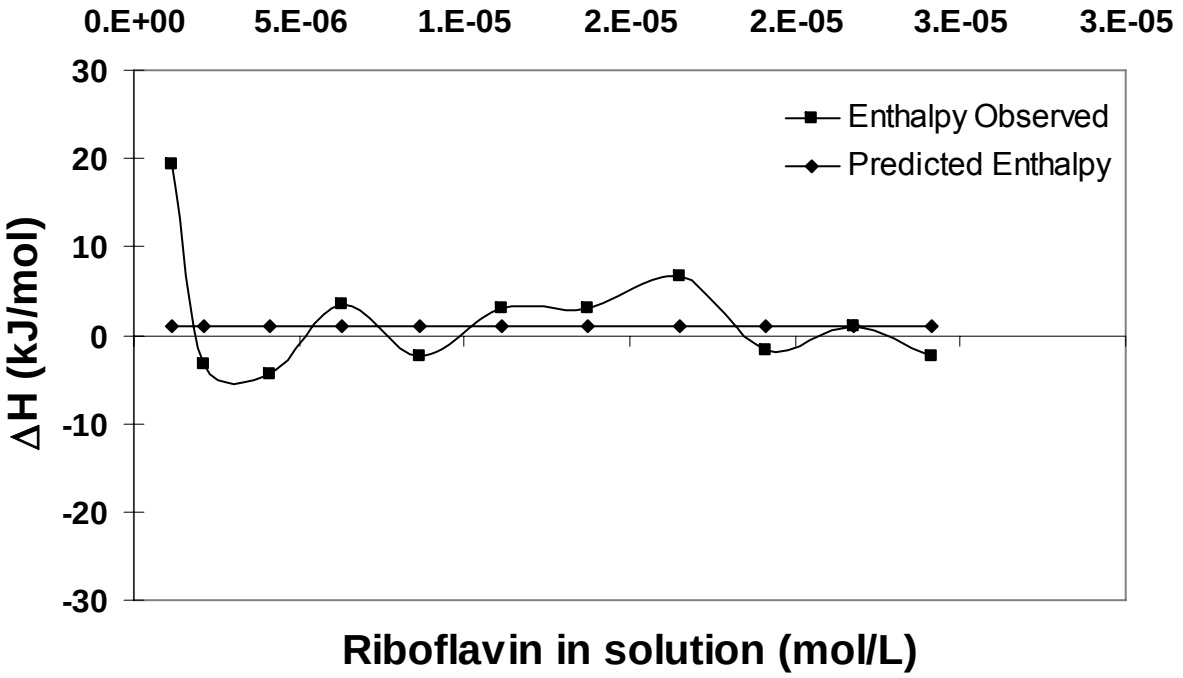


Figure 24. Enthalpy of sorption of NSP for RF. ΔHads ranged from -4.44 to 19.31 kJ/mol with a predicted value of 1.1 kJ/mol.

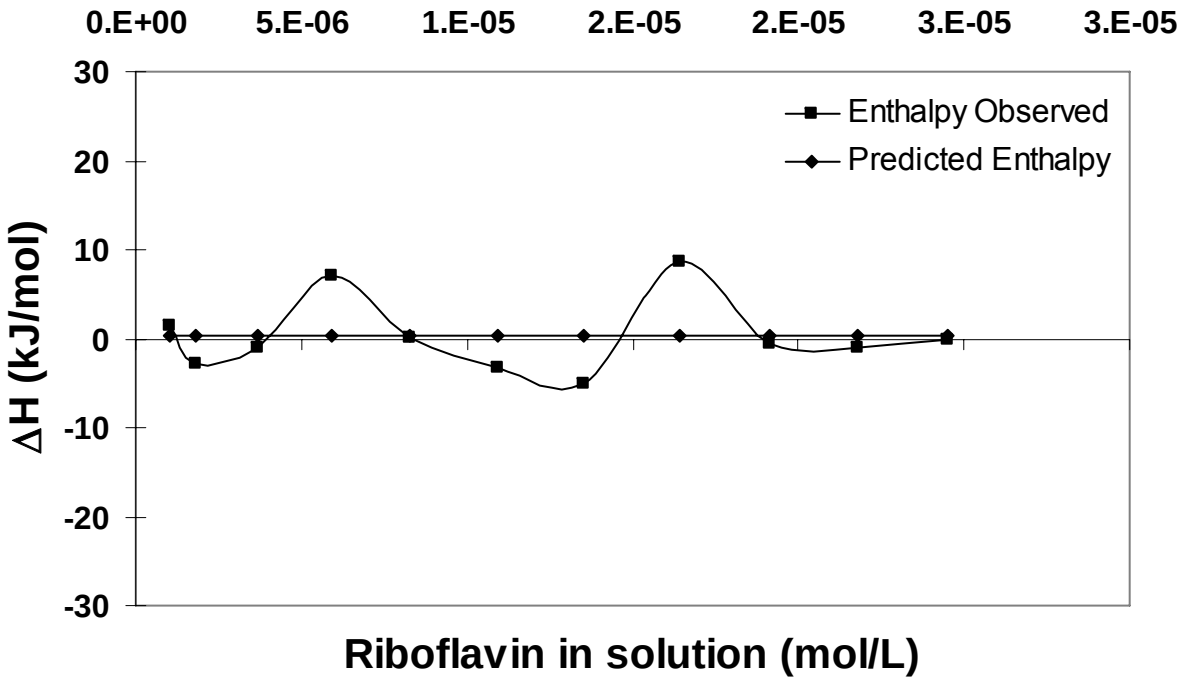


Figure 25. Enthalpy of sorption of SWy-2 for RF. ΔHads ranged from -5.03 to 8.57 kJ/mol with a predicted value of 0.3 kJ/mol.

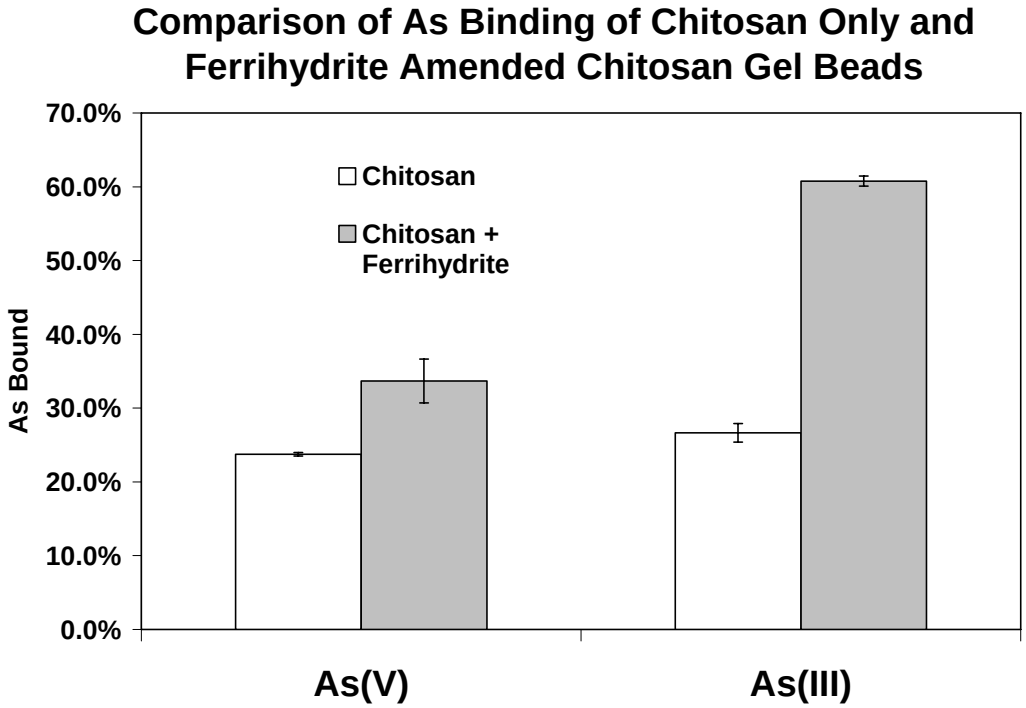


Figure 26. As (III) and As(V) sorption by chitosan and chitosan/ferrihydrite gel beads. Chitosan only beads bound 26.7% and 23.7% of As(III) and As(V), respectively, while the chitosan/ferrihydrite beads bound 60.77% and 33.7% of As(III) and As(V), respectively. Error bars represent ± 1 standard deviation.

CHAPTER III

IN VITRO EVALUATION AND MODELING OF FERRIHYDRITE AS AN ENTEROSORBENT FOR ARSENIC FROM CONTAMINATED DRINKING WATER*

Introduction

Ferrihydrite has been shown to be an effective sorbent of As at a pH ranging from 4 to 10 (*56*). To be an effective enterosorbent for As, ferrihydrite must be able to sorb both As(III) and As(V) at even lower pH's and maintain this interaction throughout the more neutral pH conditions found in the intestine. Since acid dissolution could potentially be an issue with its application as an enterosorbent, ferrihydrite must be tested to ensure that its inclusion in the diet would not expose an animal or human to toxic levels of Fe.

Currently, ferrihydrite has not been tested for sorption of As at pHs approaching that of the stomach (about 2), but Beak et al. demonstrated that As(V) that was sorbed to ferrihydrite remained bound throughout a simulated stomach and intestinal phase (*66*). Measured iron levels in solution showed that dissolution of ferrihydrite was low during the stomach phase, and no dissolution occurred in the intestinal phase of their experiments (*66*). Other authors

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* Portions of this chapter are reproduced in part with permission from Taylor JF, Robinson A, Johnson N, Marroquin-Cardona A, Brattin B, Taylor R, Phillips TD. In vitro evaluation of ferrihydrite as an enterosorbent for arsenic from contaminated drinking water. *Environ Sci Technol*. **2009** Jul 15;43(14):5501-6.

investigating the bioavailability of As from soil showed that As had limited bioavailability due to potential interactions with soil minerals and metal oxides (*64, 13, 65*). In a study similar to Beak et al. (*66*), results from a simulated GI tract also correlated well with results in pigs fed As contaminated soils (*110*).

The limited bioavailability of As from soils combined with the capacity of ferrihydrite to sorb As indicates that ferrihydrite may represent a possible enterosorbent for As from contaminated drinking water. To test its potential as an enterosorbent we wanted to further delineate its As sorbing capacity in water using isothermal analysis. Ferrihdyrite was also tested for sorption of As, using a similar gastro-intestinal model previously mentioned (*66, 110*). Ferrihydrite was tested for its ability to rescue *Hydra vulgaris*, a small aquatic organism that is very sensitive to As, from toxicity while evaluating ferrihydrite's general safety in the assay.

Chemical Reagents and Sorbents

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) and were at least ACS reagent grade. 2-line ferrihydrite was prepared according to Schwertmann and Cornell, 2000 (*50*). IPF was obtained from BASF. All iron oxides were air-dried in a fume hood and ground and sieved to <100 *μ*m. Iron oxides were verified by X-ray diffraction, and their surface areas were measured after degassing at 110°C for 2 h (*56*) using a multipoint BET analysis with N_2 adsorption on a Micromeritics ASAP 2020.

Arsenic and Iron Analysis

Sample extracts were diluted 1:1 with a solution containing deionized

water, 2% nitric acid (v/v), and 10% hydrochloric acid (v/v) in order to match the sample matrix to the standards used to calibrate the instrument. Samples were then analyzed for As and Fe using a Spectro CirOS axial ICP-OES (Spectro AI, Fitchburg, MA) employing a modified version of USEPA Method 200.7 (*107*). The diluent was analyzed as an independent sample, which showed no traces of As or Fe at the levels measured in the samples. The instrument was calibrated using a series of standards (As 100 ppm, Fe 500 ppm) at approximately twice the concentration in solution observed in the highest test sample for As and above all Fe samples. The calibration was verified against independent standards and the calibration blank before the analytical run, approximately every ten samples during the run, and at the end of the analytical run. Ytterbium was employed as an internal standard.

Arsenic Isotherm Procedure

Isotherms were run using modifications of methods previously reported (*56, 69*). Stock solutions of 125 ppm As(III) and As(V) were prepared in water from sodium metarsenite and sodium arsenate, respectively. The final pH of each solution was adjusted to 7. From these stock solutions of As(III) or As(V), a series of dilutions was added to 15 mL polypropylene tubes along with 1 mL of a 5 mg/mL suspension of sorbent for a final volume of 5 mL and final sorbent concentration of 1 mg/mL. Final concentrations of the respective arsenic species were 100, 50, 25, 10, 5, 2.5, and 1 ppm. Each dilution was prepared in triplicate. The tubes were then agitated at 1000 rpm on a shaker at 25 °C for 4 h. They were then centrifuged at 2500 rpm for 20 min, and 2.5 mL of the supernatant was

saved for arsenic analysis via ICP-OES. Binding data were fit to the Langmuir equation using Table 2 Curve software (*70*).

Adult Hydra Bioassay

The adult Hydra bioassay was carried out as previously described (*111, 112*). *Hydra vulgaris* were obtained as a gift from E. Marshall Johnson, Jefferson Medical College (Philadelphia, PA) and were cultured in shallow trays containing media consisting of 1.0mM calcium chloride, 0.45 mM TES buffer, and 0.012 mM EDTA, pH 7 at 18 °C. These conditions ensure asexual reproduction of Hydra, which aids in the reliability and reproducibility of this assay. For assays, only adult Hydra were used. For each concentration of As(III) or As(V), 4.0 mL of media, with or without As, were placed in a small Petri dish at 18 °C; all assays with As were carried out without EDTA in the media. Adult Hydra were observed for signs of toxicity at 0, 4, 20, 28, 44, 68, and 92 h. The tulip stage has been validated as the toxic end point of the assay (*111, 112*). The minimal effective concentration (MEC) at 92 h was determined for As(III) and As(V) through a series of assays, consisting of three phases. The first phase involved exposing adult Hydra to whole log concentrations of each As specie to determine the range of toxicity apparent within 92 h. The lowest concentration of As resulting in the toxic end point, MEC, was carried forward to the second phase of testing. In phase II of testing, the highest and lowest concentrations of As were within one log unit of one another. The MEC from the second phase of testing was used as the highest concentration in the phase III testing. The results from the phase III test were used to confirm those of the previous assays. To evaluate ferrihydrite's

ability to protect Hydra, 0.25% ferrihydrite was added to each dish 24 h prior to the addition of Hydra, and the assay was carried out, as previously indicated.

Arsenic Adsorption to Ferrihydrite in a Simulated GI Model

As adsorption on ferrihydrite was evaluated in a simulated GI model (Figure 26). We used a previously described method with slight modifications (*66, 110*). To simulate the binding of As to ferrihydrite in the stomach, 1.5 g of ferrihydrite was added to 600mL of water in a water-jacketed beaker at 37 °C containing 0, 10, 25, 50, or 100 ppm As(V) or As(III) at pH 1.8 using concentrated HCl. The solution was stirred with an overhead stirrer at approximately 100 rpm, while argon was bubbled through the solution. The total time for the stomach phase was 2 h. To simulate the conditions in the intestine, the pH of the solution was raised to 6.5 by the addition of saturated $NaHCO_3$. The total time in the intestine phase was 4 h with the total time for both phases equaling 6 h. Aliquots (40 mL) were taken at the end of each phase and centrifuged at 10,000 rpm for 10 min, and the supernatant was filtered through a 0.45 μm nylon filter and analyzed for Fe and As by ICP-OES.

Results and Discussion

Isotherms for As(III) and As(V) on ferrihydrite illustrated its ability to sorb As (Figure 27). Ferrihydrite had a maximum adsorption capacity (Q_{max}) and affinity (K_d) of 1.288 mol/kg and 2.7×10^3 and 0.745 mol/kg and 5.27×10^4

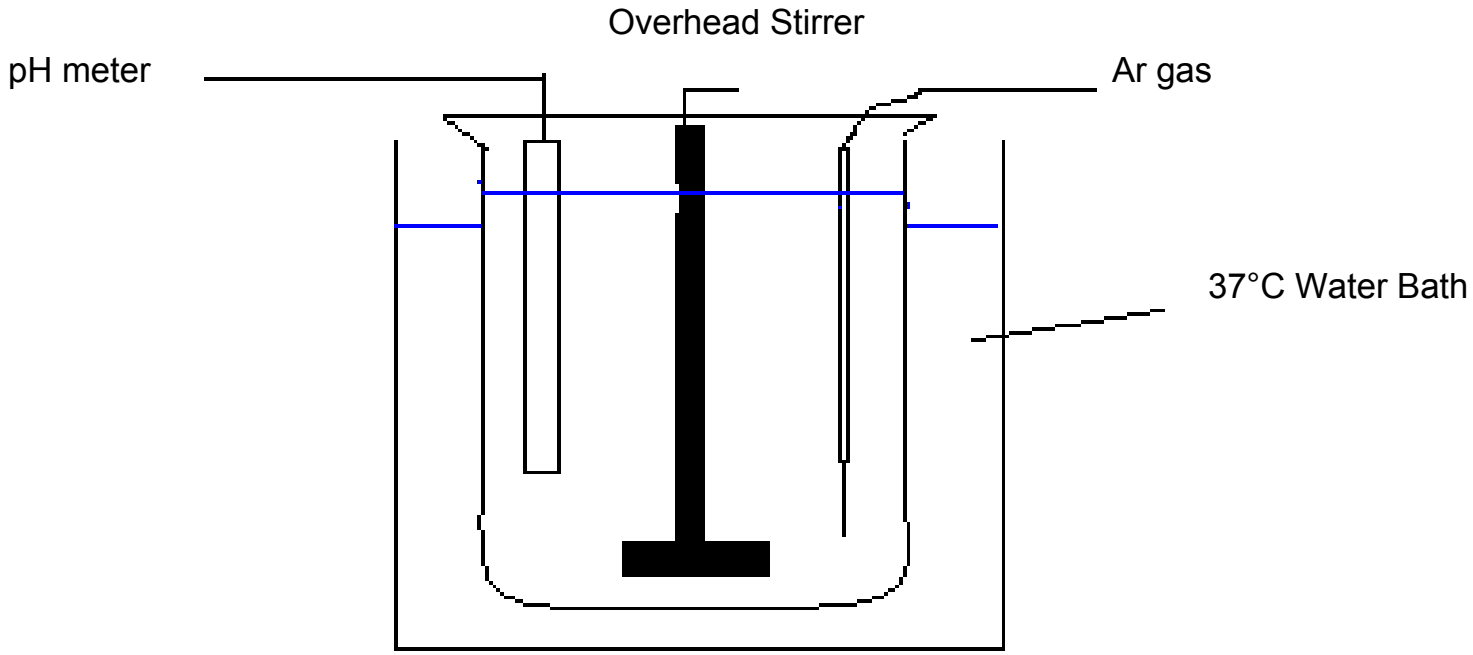

Figure 27. Apparatus for the simulated gastrointestinal model.

mol/kg for As(III) and As(V), respectively (Figure 28). IPF had a Q_{max} and K_d of 0.452 mol/kg and $1.08*10^5$ and 0.252 mol/kg and 2.35×10^5 for As(III) and As(V) respectively (Figure 29). Ferrihydrite's high capacity for As adsorption has been demonstrated previously (*56*). As(V) and As(III) both form bidentate complexes on the surface of ferrihydrite (*113-116*). The high sorption capacity of ferrihydrite is due to a high surface area, calculated as 273 m^2/g by multipoint BET. IPF had a surface area of 193 m^2/g, which could partially explain its diminished capacity as compared to ferrihydrite synthesized in our lab. The surface area measurements are consistent with previous characterization of this material indicating that the surface area of synthetic ferrihydrite ranges between 200-320 m^2/g (*50*). Theoretically, the surface area could be approximately 600 m^2/g, but ferrihydrite is typically aggregrated during drying (*50*). X-ray diffraction patterns confirm that both ferrihydrite produced by our lab and IPF are 2-line ferrihydrite, as no other minerals could be detected. Since the goal of these experiments was to find a suitable and cost effective sorbent, ferrihydrite produced in our lab was air-dried, which is a method that requires minimal attention and no expensive equipment. IPF was chosen for inclusion in these tests to represent an industrially-produced product that is already on the market.

Due to the pH-dependent nature of the As-Fe binding reaction, As binding to ferrihydrite was further demonstrated using a simulated gastro-intestinal (GI) model. This GI model has been used to evaluate the potential bioavailability of As from contaminated soil and As-loaded ferrihydrite (*66, 110*). Moreover, it has

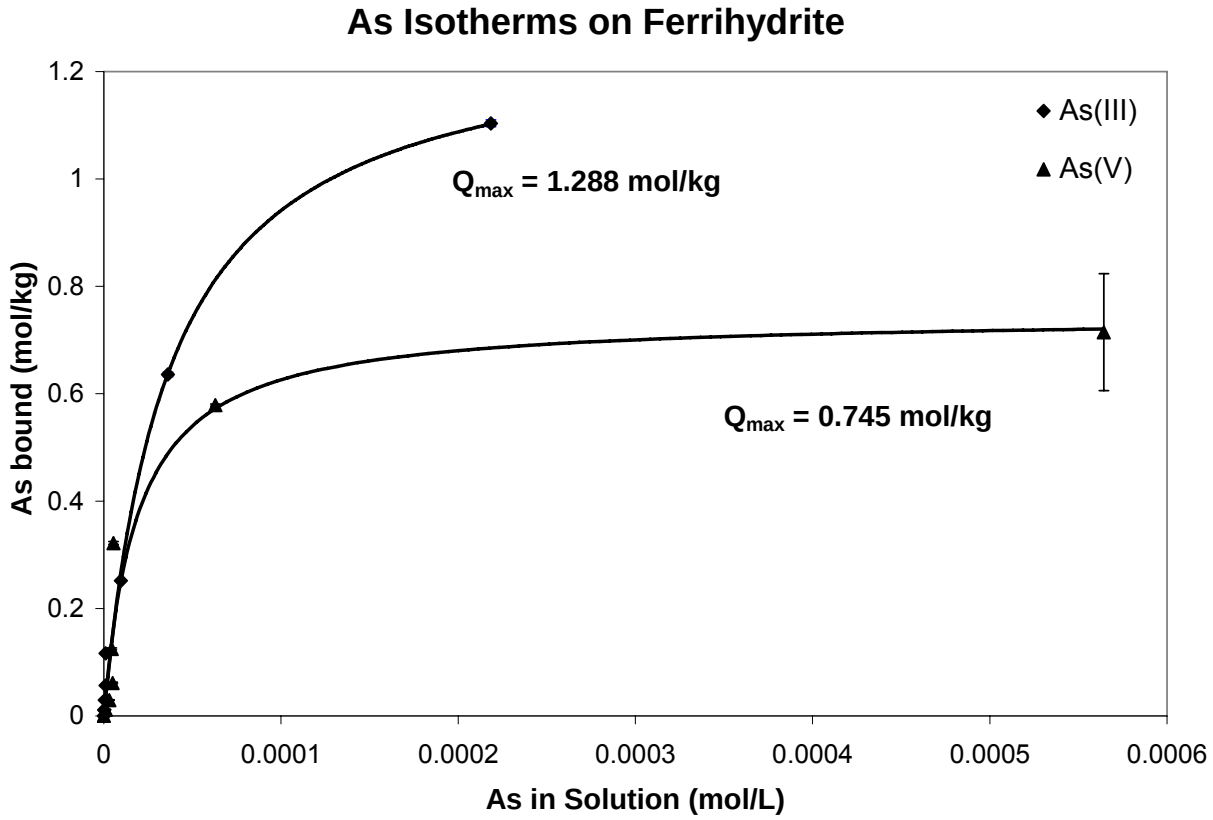

Figure 28. Isotherms performed at initial pH 7 on ferrihydrite. Isotherms performed at initial pH 7 verified ferrihydrite's high sorption capacity for both arsenate and arsenite. The high capacity of ferrihydrite for As(III) and As(V) is demonstrated by Q_{max} values of 1.288 and 0.745 mol/kg, respectively. Error bars represent ± 1 standard deviation.

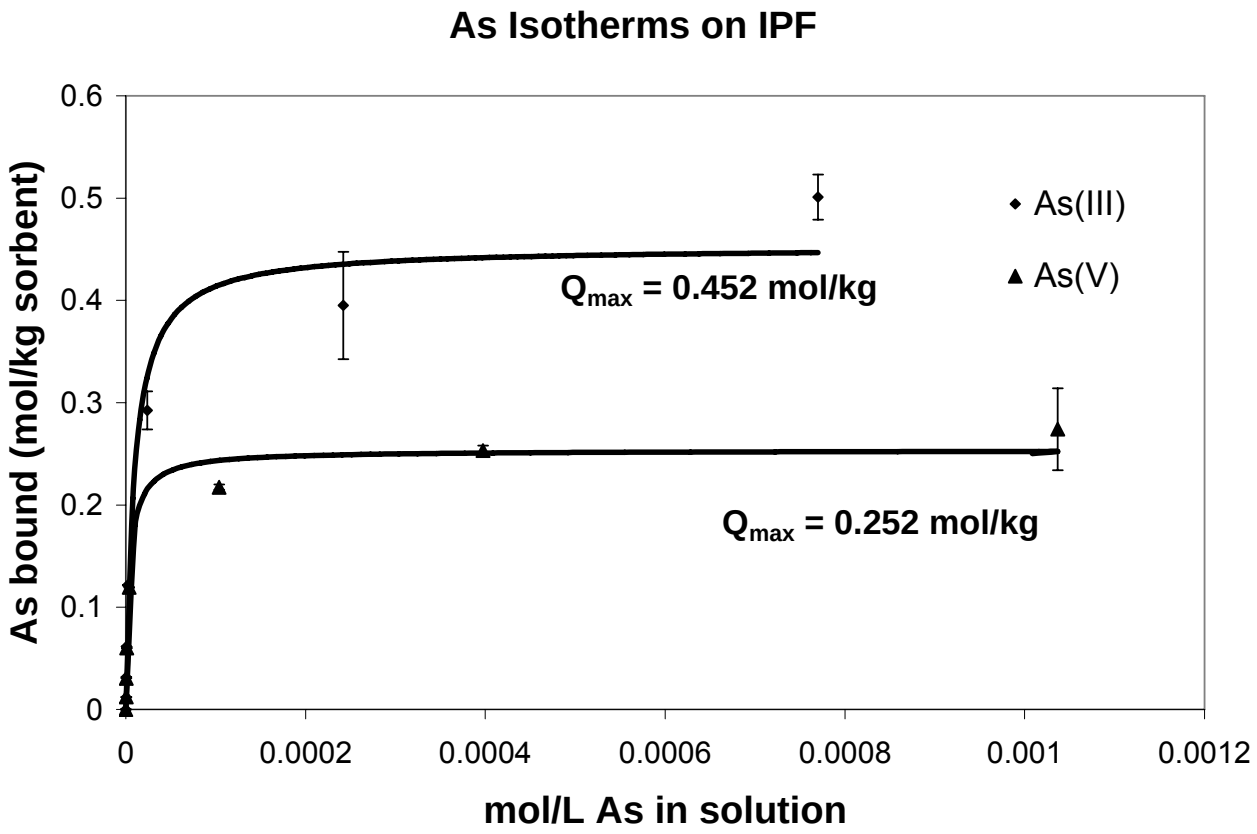

Figure 29. Isotherms performed at initial pH 7 on IPF. Isotherms performed at initial pH 7 verified IPF's high sorption capacity for both arsenate and arsenite. The high capacity of ferrihydrite for As(III) and As(V) is demonstrated by Q_{max} values of 0.452 and 0.252 mol/kg, respectively. Error bars represent ± 1 standard deviation.

been positively correlated with in vivo results in pigs (*110*). Experiments were conducted to evaluate ferrihydrite's ability to sorb As from the GI tract, to simulate an exposure to contaminated drinking water with subsequent or prior treatment with ferrihydrite as an enterosorbent. The results showed that ferrihydrite retained significant affinity for both As(III) and As(V) with Q_{max} values of 0.200 mol/kg and 0.186 mol/kg, respectively, in the simulated stomach (Figure 30). IPF was also effective in removing As from the simulated stomach as demonstrated by the maximum binding values of 0.311 mol/kg and 0.246 mol/kg for As(V) and As(III), respectively (Figure 31). These values were lower than what was found in isotherms for ferrihydrite, which could be a result of the optimal pH of 4 for As(V) binding, while the optimal pH for As(III) is above 7 (*56*). Small changes in sorption could have resulted from As(III) being oxidized to As(V) by the conditions of the simulated stomach. However, oxidation of As(III) in the simulated stomach (pH 1.8 under anoxic conditions) was unlikely, given the duration of our study, i.e., 2 h. Nakazawa and Hareyama noticed no observable As(III) oxidation in aqueous media at pH 1.8 after 5 days (*117*).

Q_{max} values for As binding to ferrihydrite in the simulated intestine were 0.276 mol/kg and 0.248 mol/kg for As(III) and As(V), respectively (Figure 32). Q_{max} values for IPF were 0.344 mol/kg and 0.365 mol/kg for As(III) and As(V), respectively (Figure 33). Raven et al. showed that ferrihydrite had similar affinities for both As(III) and As(V) from pH 6-7.5 (*56*). These values are also similar to those found for mycotoxin binders which have been shown to be

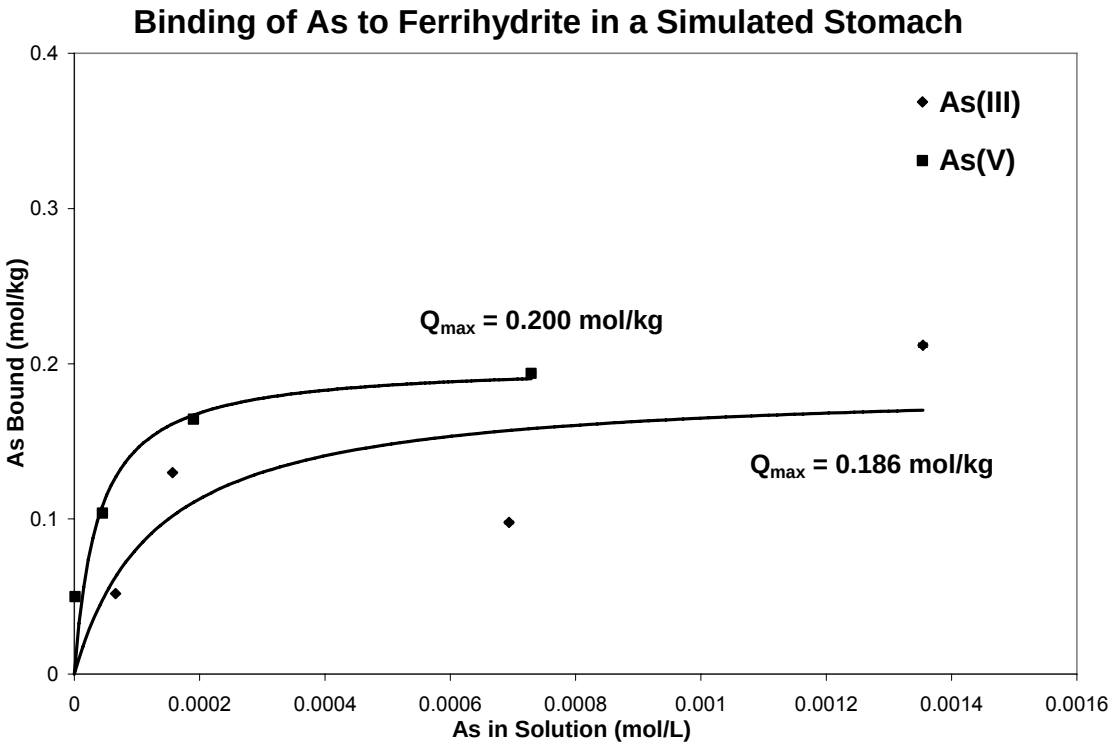

Figure 30. Binding of As to ferrihydrite in a simulated stomach. Ferrihydrite sorbed both As(V) and As(III) at a high capacity in a simulated stomach shown by Q_{max} values of 0.200 and 0.186 mol/kg, respectively. These results confirm that ferrihydrite retains a high capacity at low pH and can significantly sorb As in a simulated stomach. Error bars represent ± 1 standard deviation.

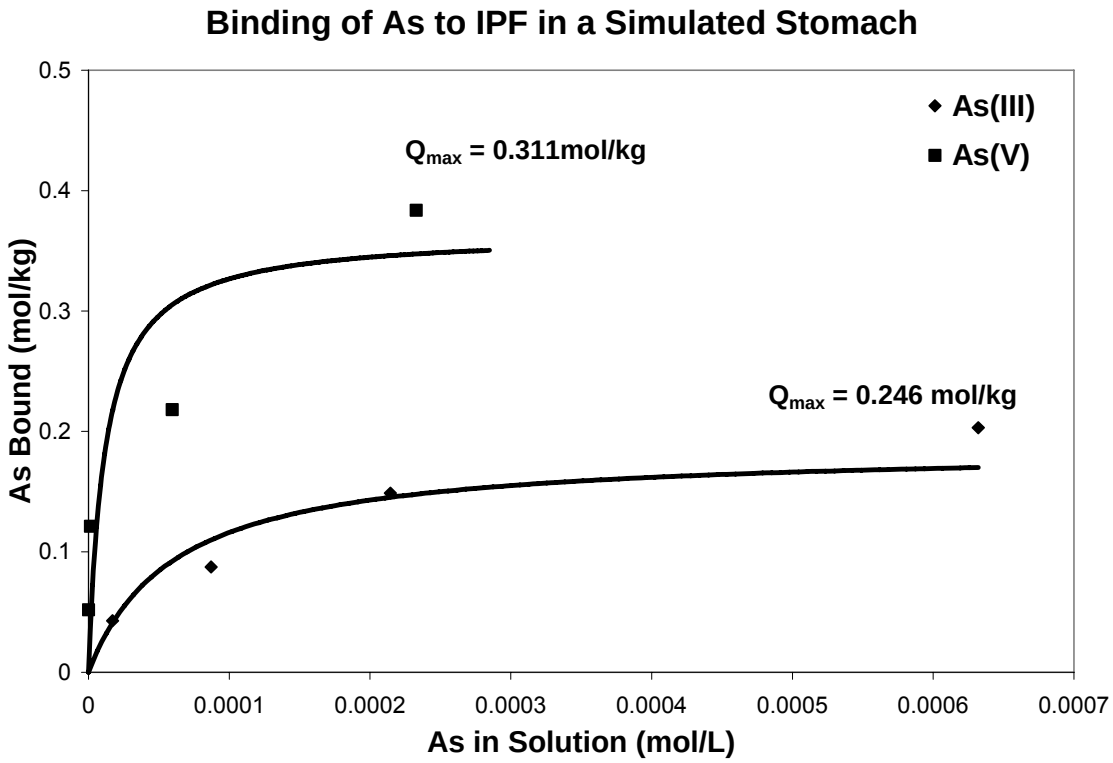

Figure 31. Binding of As to IPF in a simulated stomach. IPF sorbed As(V) and As(III) at a high capacity in a simulated stomach shown by Q_{max} values of 0.246 and 0.311 mol/kg, respectively. These results confirm that IPF retains a high capacity at low pH and can significantly sorb As in a simulated stomach. Error bars represent ± 1 standard deviation.

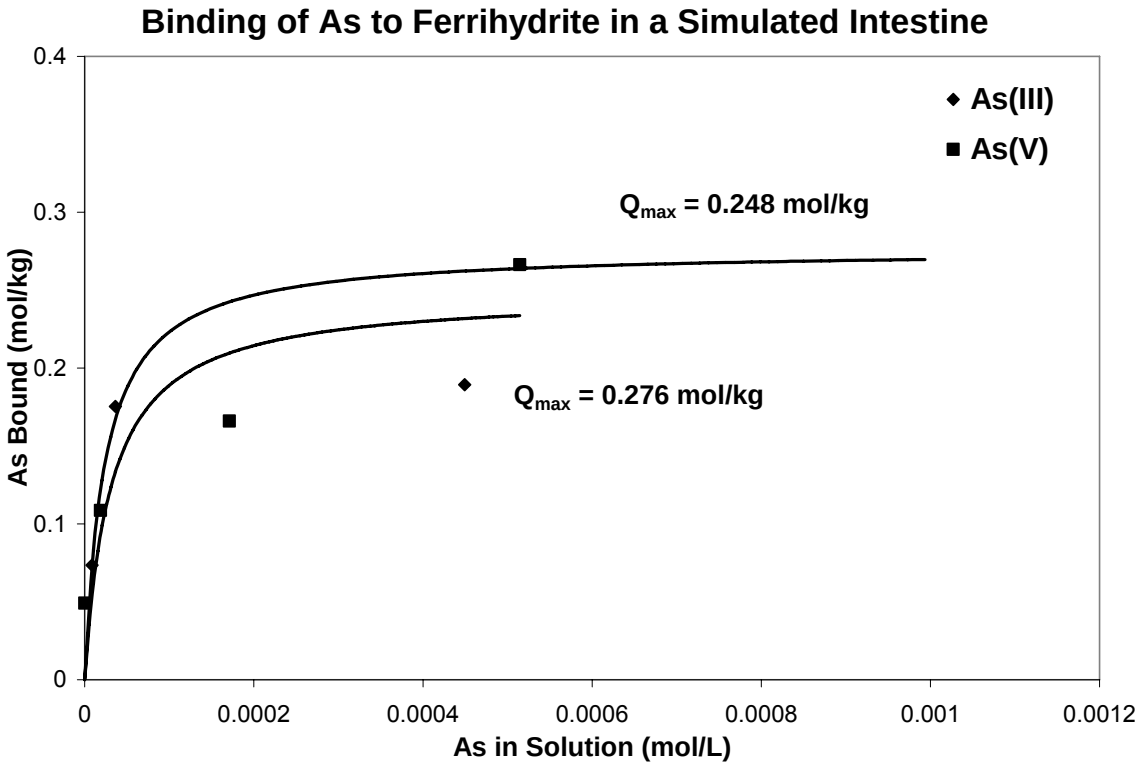

Figure 32. Binding of As to ferrihydrite in a simulated intestine. Ferrihydrite sorbed both As(V) and As(III) at a high capacity in a simulated intestine shown by Q_{max} of 0.248 and 0.276 mol/kg, respectively. Q_{max} values for both As species were higher in the simulated intestine than the simulated stomach, which suggests that As(III) and As(V) would remain sorbed throughout the GI tract offering protection from As found in contaminated drinking water. Error bars represent ± 1 standard deviation.

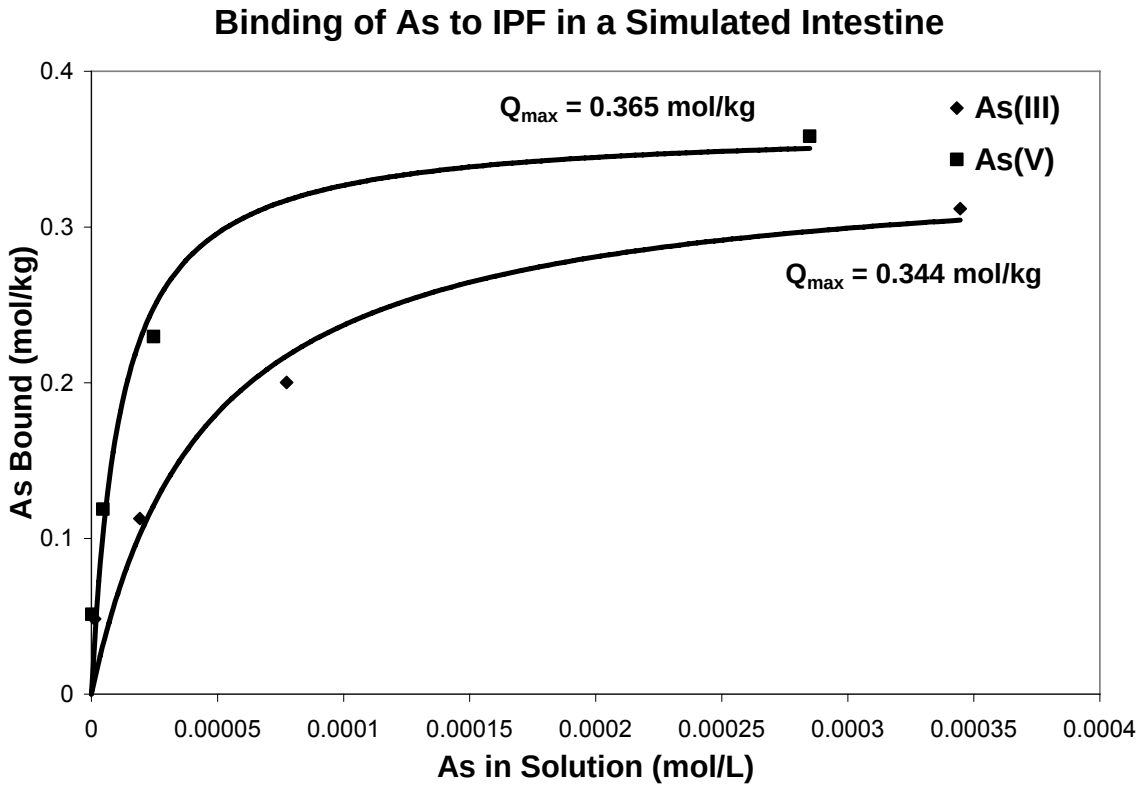

Figure 33. Binding of As to IPF in a simulated intestine. IPF sorbed both As(V) and As(III) at a high capacity in a simulated intestine shown by Q_{max} of 0.344 and 0.365 mol/kg, respectively. Q_{max} values for both As species were higher in the simulated intestine than the simulated stomach, which suggests that As(III) and As(V) would remain sorbed throughout the GI tract offering protection from As found in contaminated drinking water. Error bars represent ± 1 standard deviation.

effective in vivo (*69, 102, 118*). These data show that ferrihydrite may sorb both As species in the stomach, and maintain this sorbed As throughout the GI tract.

Along with As, the quantity of Fe that passed through the 0.45 *μ*m filter was measured. Similarly to Beak et al. (*66*), we chose to use a 0.45 *μ*m filter to mimic the 0.5 *μ*m size cutoff of particles capable of entering epithelial cells. This would approximate the Fe exposure to cells that is either dissolved Fe (likely in the form of Fe^{3+}) or colloidal ferrihydrite remaining in suspension after centrifugation. This measurement is a likely representation of the maximum Fe level that would be bioavailable from ferrihydrite. For ferrihydrite produced in our lab, the levels of Fe in the simulated stomach decreased with increasing As(V) concentration, similar to what was previously reported (*66*) (Figure 34); however, there was no clear trend of Fe concentration with respect to increasing As(III) concentration for ferrihydrite (Figure 35). For IPF, there was a clear trend for reduced Fe levels with increasing As(V) concentrations (Figure 36), while As(III) showed a weaker trend (Figure 37). The maximum Fe levels seen in the stomach phase for ferrihydrite reached 7.57 ppm and 8.97 ppm with initial As(V) concentration of 10 ppm and initial As(III) concentration of 10 ppm, respectively. Max level of Fe seen in IPF experiments was at 0 ppm As and was found to be 18.01 ppm. Concentrations of Fe seen in the simulated intestine were very low for all concentrations tested and for both As(III) and As(V) with both ferrihydrite and IPF. The highest Fe concentration measured in the intestine was for ferrihydrite or IPF was 0.12 ppm. The recommended daily intake of Fe is 18 mg,

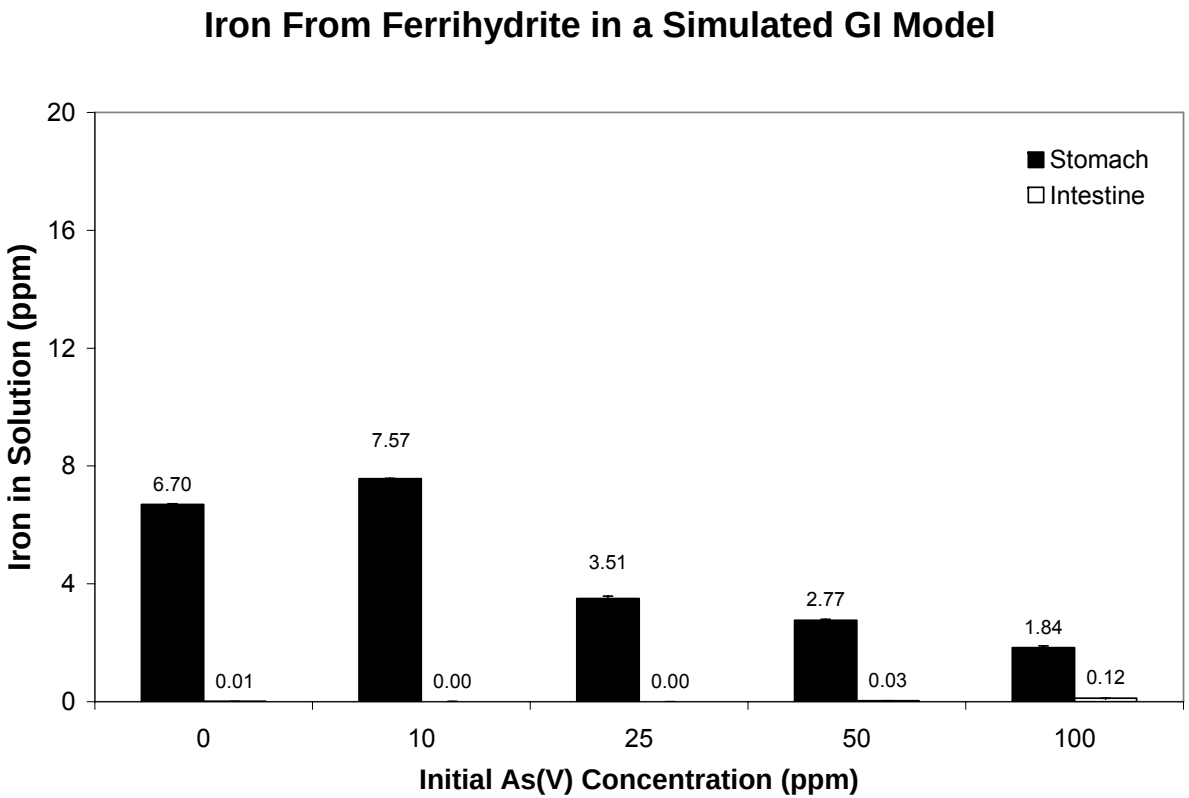

Figure 34. Iron from ferrihydrite in a simulated GI model at varying As(V) concentration. In the simulated stomach, measured iron generally decreased as initial As(V) concentration increased suggesting that the increased surface coverage of As(V) on ferrihydrite offered protection from acid dissolution. Iron levels were low throughout the simulated intestine.

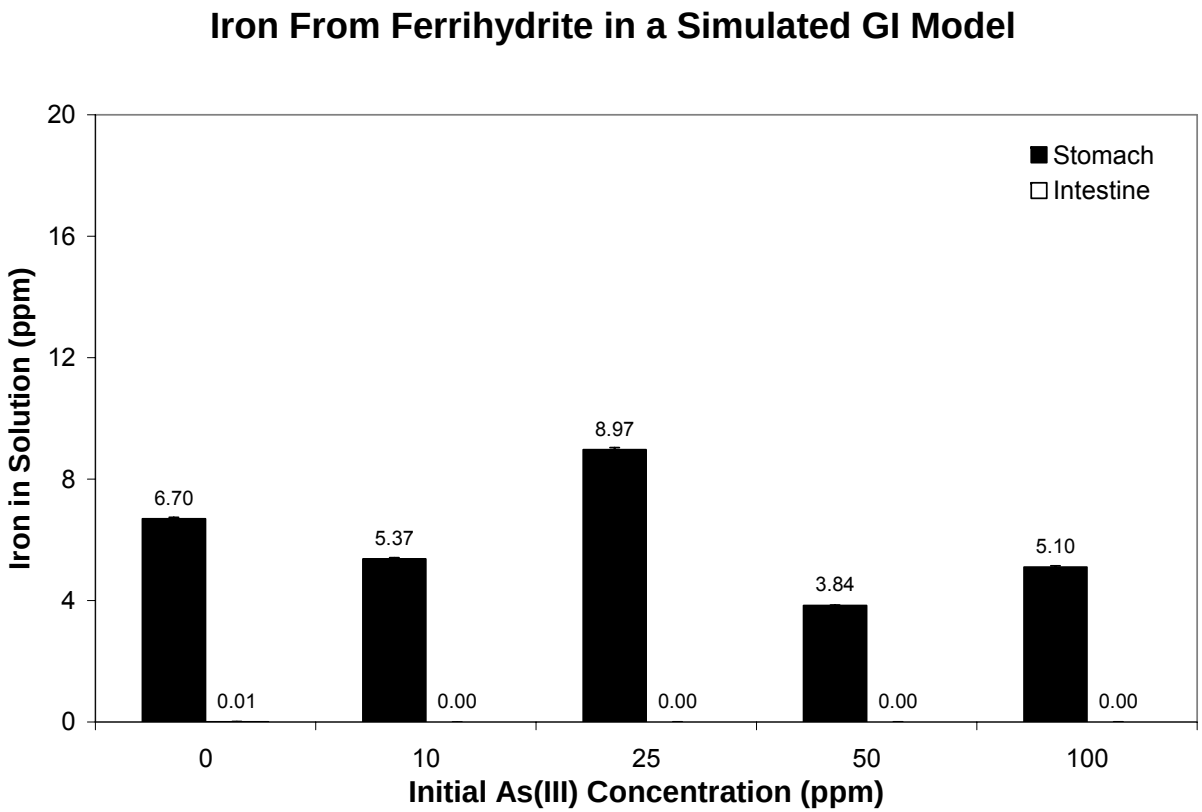

Figure 35. Iron from ferrihydrite in a simulated GI model at varying As(III) concentration. In the simulated stomach, measurable iron was found with all initial As(III) concentrations with no clear pattern of iron concentration. Iron in solution was very low in the simulated intestine at all initial As(III) concentrations.

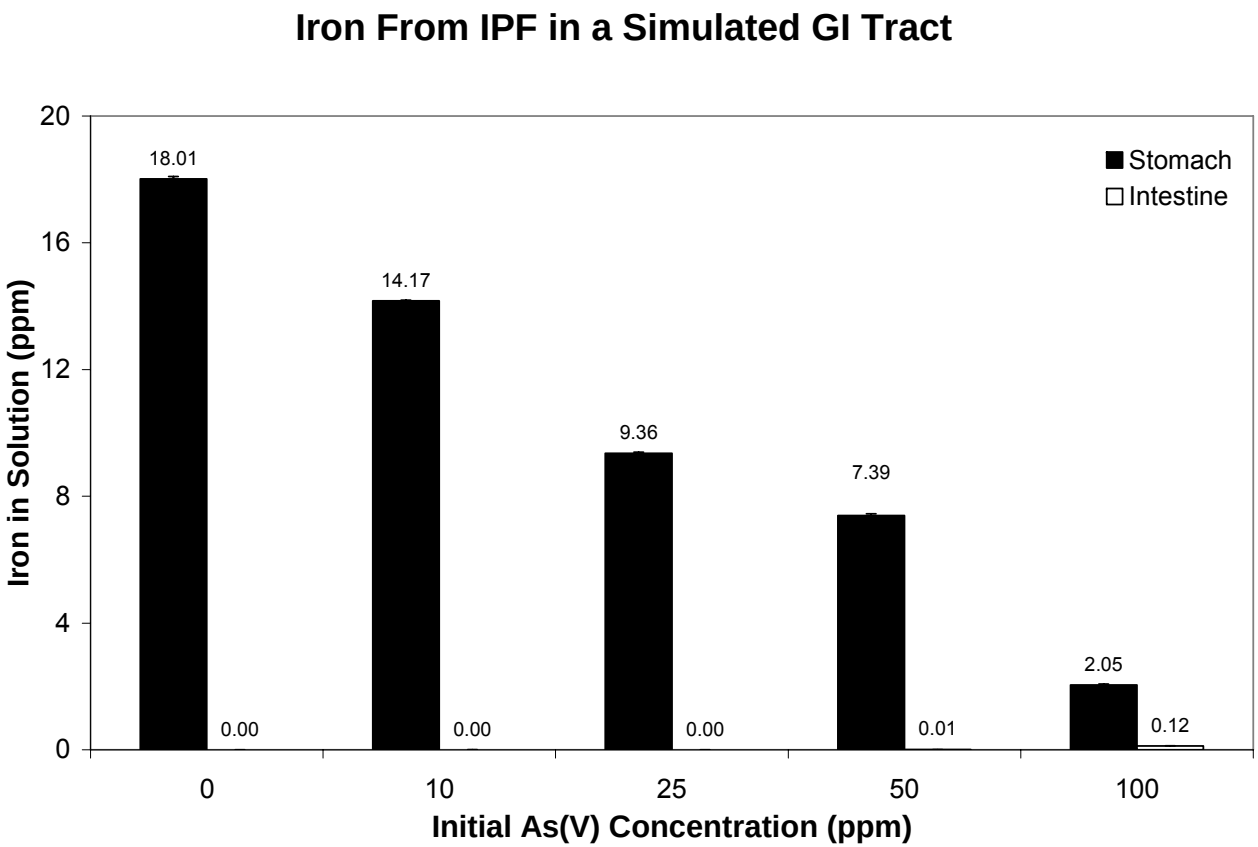

Figure 36. Iron from IPF in a simulated GI model at varying As(V) concentration. In the simulated stomach, measured iron generally decreased as initial As(V) concentration increased, suggesting that the increased surface coverage of As(V) on ferrihydrite offered protection from acid dissolution. Iron levels were low throughout the simulated intestine.

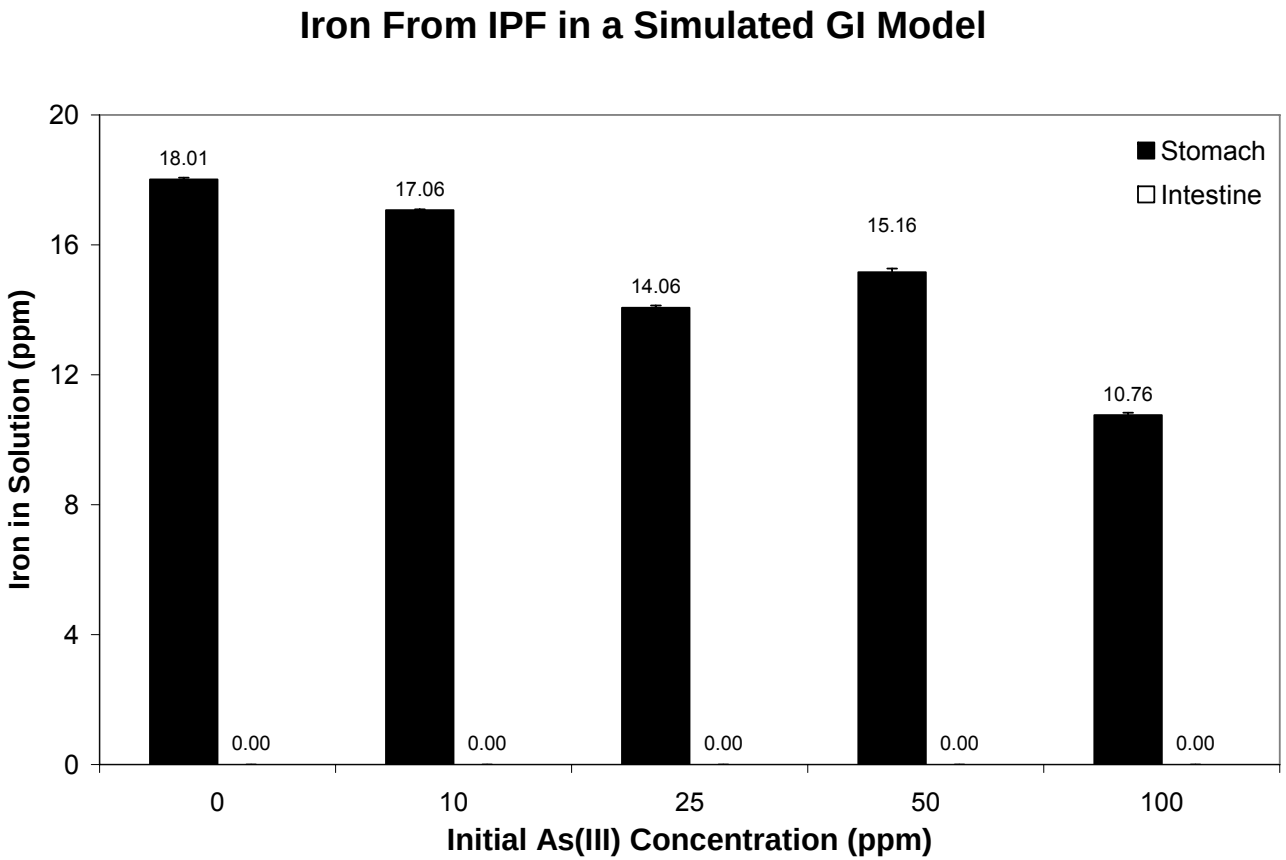

Figure 37. Iron from IPF in a sumlated GI model at varying As(III) concentration. In the simulated stomach, measurable iron was found with all initial As(III) concentrations with an overall trend of protection of acid dissolution as As concentrations increased. Iron in solution was very low in the simulated intestine at all initial As(III) concentrations.

with the maximum tolerable daily Fe intake being 40 mg for children and 45 mg for adults (*81*). The maximum expected Fe available from each of our tests with a total of 1.5 g of ferrihydrite was 5.4 mg (Table 8). For IPF, the maximum expected Fe available from ingestion of 1.5 g was 10.8 mg (Table 9). Even for 3 g of either per day, the expected bioavailable iron would be below tolerated doses in all healthy individuals. The Fe levels measured in the simulated intestine from both ferrhydrite and IPF show how poorly soluble Fe^{3+} is at higher pH values.

The World Health Organization considers Fe deficiency the number one nutritional disorder in the world (*90*). The dose (1.5 g) of ferrihydrite was chosen for the GI model based on prior work with NovaSil clay in an aflatoxin exposed group from Ghana (*79*). This ferrihydrite level throughout the GI model experiments represented the dose if enterosorption therapy is given twice daily for a total of 3 g/day. Any enterosorption therapy including ferrihydrite would have to be closely monitored, as some individuals are sensitive to Fe or suffer from hemachromatosis, which could cause a toxic buildup of excess iron (*54*). However, for the population that this enterosoption therapy with ferrihydrite is intended, any additional bioavailable Fe will likely be beneficial. Vitamin A helps mobilize Fe from storage sites, and inadequate intake of vitamin A is also a problem in developing countries (*96, 97*).

Table 8. Fe from ferrhydrite measured in the simulated stomach using the GI model. Values include the total iron measured from 1.5g ferrihydrite, with extrapolated values for a 3g/day dose.

As Specie	Initial As (ppm)	mg Fe from 1.5g	mg Fe from 3g	% DV[a] from 3g	% of Tolerable Intake[b] from 3g
	0	4.0	8.0	44.6%	20.1%
As(III)	100	3.06	6.1	34.0%	15.3%
	50	2.30	4.6	25.6%	11.5%
	25	5.38	10.8	59.8%	26.9%
	10	3.22	6.4	35.8%	16.1%
As(V)	100	1.10	2.2	12.2%	5.5%
	50	1.66	3.3	18.4%	8.3%
	25	2.10	4.2	23.4%	10.5%
	10	4.54	9.1	50.5%	22.7%

a DV = Daily Value as set by Institute of Medicine, which is 18 mg.
b Tolerable Intake for Fe from Institute of Medicine, which is 40 mg.

Table 9. Fe from IPF measured in the simulated stomach using the GI model. Values include the total iron measured from 1.5g ferrihydrite, with extrapolated values for a 3g/day dose.

As Specie	Initial As (ppm)	mg Fe from 1.5g	mg Fe from 3g	% DV[a] from 3g	% of Tolerable Intake[b] from 3g
	0	10.8	21.6	120.0%	45.0%
As(III)	100	6.45	12.9	71.7%	32.3%
	50	9.09	18.2	101.0%	45.5%
	25	8.44	16.9	93.8%	42.2%
	10	10.24	20.5	113.8%	51.2%
As(V)	100	1.23	2.5	13.7%	6.2%
	50	4.44	8.9	49.3%	22.2%
	25	5.61	11.2	62.4%	28.1%
	10	8.50	17.0	94.5%	42.5%

a DV = Daily Value as set by Institute of Medicine, which is 18 mg.
b Tolerable Intake for Fe from Institute of Medicine, which is 40 mg.

Our experiments suggest that ferrihydrite used as an enterosorbent would likely be tolerated by healthy, well nourished individuals.

Ferrihydrite was also evaluated for its ability to protect a small aquatic organism, *Hydra vulgaris*, that is highly sensitive to As. The genetic homogeneity of this organism has been strictly maintained in culture to facilitate the reproducibility of the MEC for diverse toxins including chlorinated phenols (*111*), heavy metals (*119*), estrogenic compounds (*120*), and organophosphate nerve agents (*112)*. The body wall of Hydra consists of a trilaminar structure and a hollow tube called the gastric cavity or gut. The Hydra gut contains a mouth (hypostome) and aboral pore that expels digested materials and fluids which is in equilibrium with the surrounding media and toxins. The Hydra assay has been reported to accurately predict the safety and efficacy of potential enterosorbents (*53, 121*) prior to animal studies and has been utilized along with an in vitro GI model (*122*) for screening purposes. The toxic end point of the Hydra bioassay has been validated as the tulip stage, which represents the point at which the Hydra will not recover if placed in control media. The minimal effective concentration (MEC) for As(V) and As(III) were 90 ppm and 1 ppm, respectively (Figure 38). The addition of 0.25% w/w ferrihydrite protected the Hydra at As levels of 250 ppm and 200 ppm for As(V) and As(III), respectively. For IPF, inclusion at 0.25% w/w protected Hydra up to 200 ppm for As(III) and As(V) (Figure 39). Additionally, ferrihydrite and IPF were not toxic to the hydra, unlike other sorbents that are lethal to this sensitive organism (*53, 121*). For both

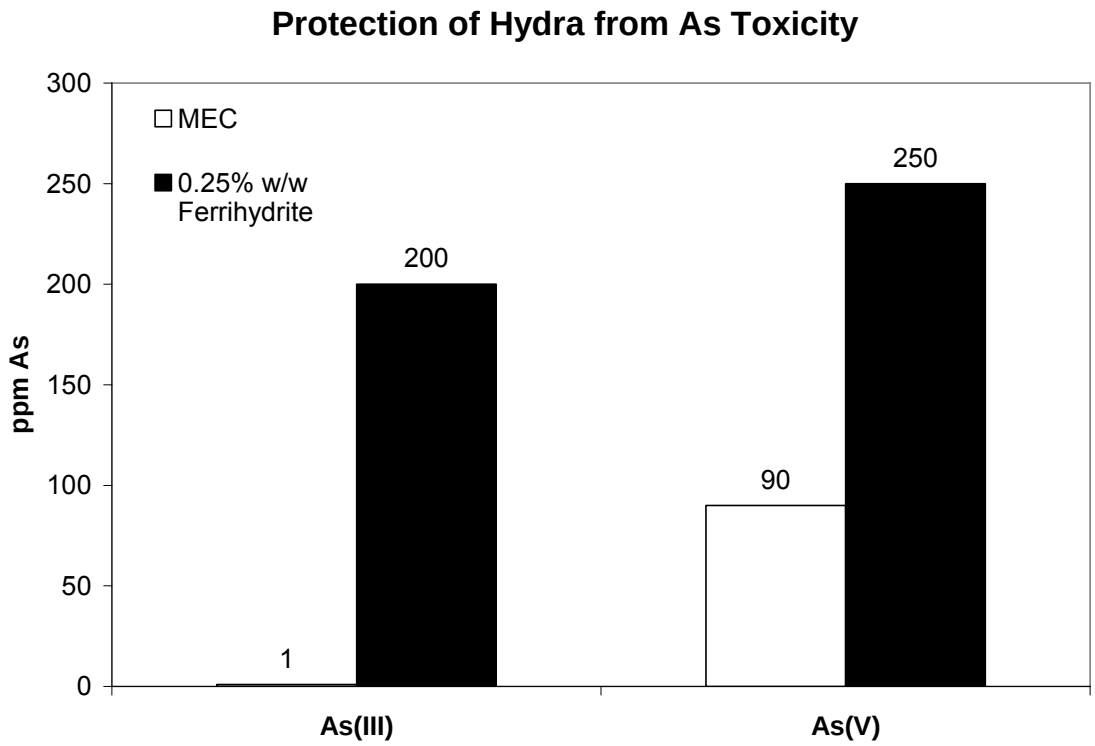

Figure 38. Protection of Hydra from As toxicity by ferrihydrite. The results show that As(III) and As(V) are lethal to Hydra at 1 ppm versus 90 ppm, respectively. Ferrihydrite protected Hydra from toxicity of As(III) and As(V) to 200 and 250 ppm, respectively, and was not toxic at 0.25% w/w.

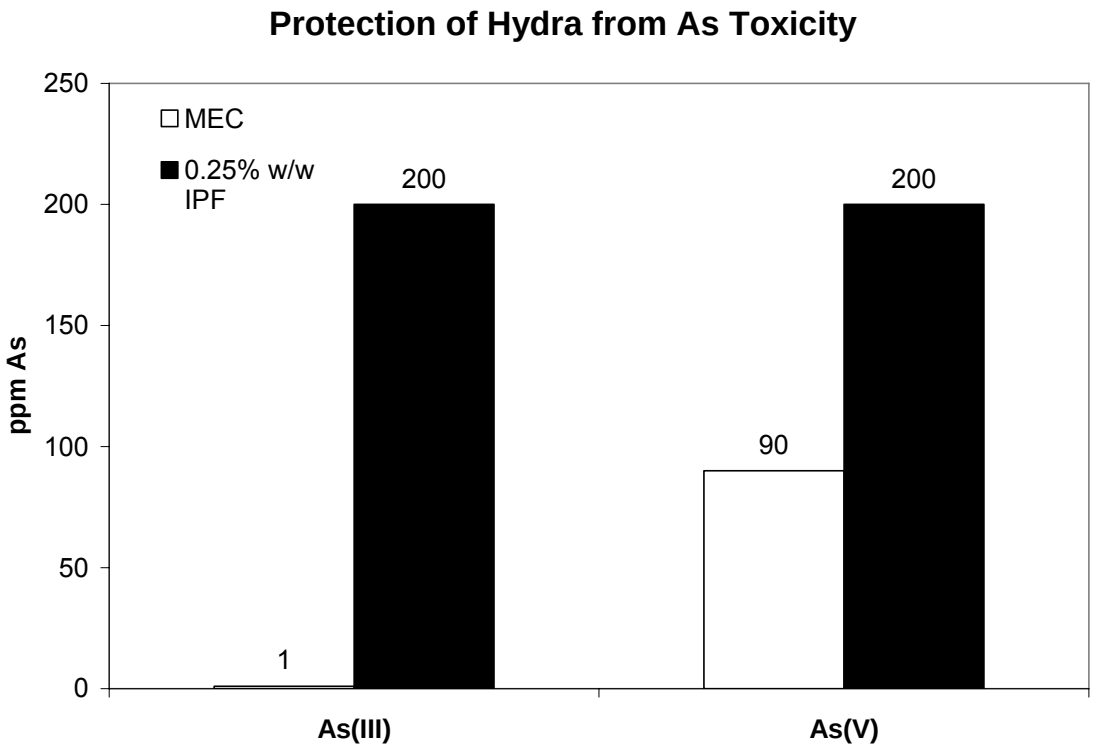

Figure 39. Protection of Hydra from As toxicity by IPF. The results show that As(III) and As(V) are lethal to Hydra at 1 ppm versus 90 ppm, respectively. IPF protected Hydra from toxicity of both species of As up to 200 ppm and was not toxic at 0.25% w/w.

ferrihydrite and IPF to protect the Hydra at 200 ppm As(III), they had to sorb over 99% of the As(III) in solution at 200 ppm, as 1 ppm was lethal to Hydra without the protection offered from ferrihydrite. These results suggest that ferrihydrite binds a very high percentage of As that could be found in contaminated groundwater. Since water can be contaminated with very high levels of As (e.g., 6.7 ppm), it is important that ferrihydrite sorb a high percentage of As in water.

The current treatments for arsenic for either acute or chronic exposure include chelation therapy with analogs of dimercaprol (BAL), oral binders such as charcoal, or agents that promote methylation and elimination of As via the urine. Other than remediating current water sources or finding alternative sources, no current treatment exists for reducing exposure to As from drinking water (*11*). A previous study found no difference in placebo and groups given 2,3-dimercaptosuccinic acid for treatment of chronic arsenicosis from drinking water (*12*). A recent toxicity profile for As by ATSDR suggested “phosphate” binders and compounds with sulfhydryl groups need further investigation for their potential as treatment for As toxicity (*11*). Although ATSDR suggested aluminum oxides as potential enterosorbents for As (*11*), ferrihydrite has been shown to be more effective at lower pH’s than aluminum oxides (*54*), and also iron oxides are generally less soluble than aluminum oxides. Our work demonstrates that ferrihydrite has a high capacity for both species of inorganic As, was effective in a simulated GI model, and protects a susceptible organism from As toxicity. Calculations based on GI models and isotherms show that 3 g of ferrihydrite can potentially protect a person drinking 2 L/day from As contaminations up to

20.9 ppm As(III) or 22.5 ppm As(V). IPF could potentially protect from up to 27.6 ppm As(III) and 34.9 ppm As(V) in drinking water. Actual exposures would likely be from a combination of As(III) and As(V), and these experiments were not designed to address which specie would be competitively favored in a dual exposure. Max protection values would likely be lower due to competition with other anions such as phosphate or sulfate found in the diet. Direct competition assays with other anions such as phosphate have shown that As is preferred on the surface of iron oxides, though competition would likely reduce overall sorption of As in an in vivo system. Previous work that focused on evaluating As bioavailability from contaminated soil suggests limited bioavailability from the soil, due in part to surface adsorption to soil minerals (*64, 65*). Concentrations of As found in contaminated wells have been shown to be as high as 6,700 ppb (*10*); which is well below the theoretical maximum protection offered from either ferrihdyrite or IPF (Table 10). This study is the first to show that ferrihydrite could offer protection from As by enterosorption, but future work is needed to verify the safety and efficacy of this sorbent in vivo.

Table 10. Theoretical maximum protection values for ferrihydrite and IPF.

		Stomach (ppm)	Intestine (ppm)	Water, pH 7 (ppm)
As(III)	2-Line Ferrihydrite	20.90	31.01	144.71
	IPF	27.64	38.65	50.78
As(V)	2-Line Ferrihydrite	22.47	27.86	83.70
	IPF	34.94	41.01	28.31

Maximum protection values are based on results of As binding in the simulated GI model and isotherms in water at pH 7. Values represent the maximum ppm of water that ferrihydrite at 3g/day will offer protection. Values are based on respective Q_{max} values and assume consumption of 2 L of water (US EPA).

CHAPTER IV

IN VIVO SAFETY AND EFFICACY OF FERRIHYDRITE AS AN ENTEROSORBENT FOR ARSENIC: SHORT TERM EVALUATION IN RODENTS

Introduction

Arsenic is a toxic metalloid that is found in groundwater primarily from natural sources but also due to industrial and agricultural processes. Arsenic in drinking water is a problem throughout the world, highlighted by exposures in Bangladesh and Taiwan. Even in the US many drinking water sources of arsenic are at, or above, the EPA limit of 10 ppb. Adverse health effects include various cancers, skin lesions, neurological effects, hypertension and cardiovascular disease, pulmonary disease, peripheral vascular disease, and increased incidence of diabetes mellitus (*2*). Inorganic arsenic is the most common and important form of arsenic found in groundwater with arsenite and arsenate as the most common forms. Arsenite is found under reducing conditions often found in groundwater, whereas arsenate is predominant under oxidizing conditions. Slow redox conversion of the two species leads to both forms being present in groundwater (*55*), and thus any treatment or filter technology must be capable of removing both As species.

Current methods for reducing exposure to arsenic in drinking water are to remediate the source or find alternate sources. Treatments for As exposure include chelating agents with analogs of dimecaprol, sorbents such as charcoal, and agents that promote methylation of arsenic and its subsequent elimination

from the body via urine (*11*). The chelating agent 2,3-dimercaptosuccinic acid was found to be ineffective in a clinical trial focusing on the treatment of chronic arsenicosis (*12*), while non-selective enterosorbents such as charcoal do not provide a safe solution for individuals chronically exposed to arsenic.

In a recent toxicological profile by the Agency for Toxic Substances and Disease Registry, “phosphate binders” were suggested for further study as potential sorbents for As ingestion (*11*). These types of agents are best characterized as enterosorbents or materials that serve to bind a specific toxin in the gastrointestinal tract thus reducing its bioavaibility and toxicity. This strategy has been targeted for a variety of toxins including the aflatoxins (and now arsenic) (*67*). In the previous chapter ferrihydrite was investigated in vitro for its potential to interact with arsenic. Ferrihydrite sorbed both arsenite and arsenate in a simulated GI tract, and it protected an Assensitive aquatic organism, *Hydra vulgaris*, from the toxic effects of arsenic. The goal of this work was to evaluate ferrihydrite’s safety and efficacy in a short term rodent model using+ Sprague-Dawley rats.

Materials and Animals

Ferrihydrite used in this study was acquired from BASF or synthesized using methods previously described (*50*). All ferrihydrite used was ground and sieved to a particle size of 100 μm and verified by XRD to be 2-line ferrihydrite. Sodium metaarsenite and sodium arsenate were obtained from Sigma and were of the highest purity available. Rats (6 per group) used were 4 week old male Sprague-Dawleys obtained form Harlan (Houston, TX). They were housed at the

Comparative Medicine Program (CMP) facilities at Texas A&M University (College Station, TX). All animal use protocols were approved by CMP.

Short Term Efficacy Study

Four week old male Sprague-Dawley rats (6 per group) were fed either a powdered control diet (Harlan Teklad 8604), or a 0.5% w/w ferrihydrite feed that was mixed thoroughly. After a one week acclimation period, rats were gavaged with 0.5 mL of 500 ppm arsenate or arsenite with and without 0.5% ferrihydrite. The rats were then transferred to metabolism cages (Nalgene, model MTB-0350), and urine was collected at 24 and 48 hours post gavage. Urine was analyzed for total As via EPA method 7062 at Columbia Analytical Services in Kelso, WA and analyzed for urinary creatinine at the Texas Veterinary Medical Diagnostic Lab (College Station, TX) using an auto-analyzer.

Short Term Safety Study

In the short term safety studies, rats (6 per group) were fed either a control diet (Harlan Teklad 8604) or control diet plus 0.5% IPF for two weeks. Subsequently the rats were euthanized by CO_2 asphyxiation, blood was collected from the heart, and all major organs were observed for gross lesions. Blood was analyzed at the Texas Veterinary Medical Diagnostic Lab (College Station, TX) for serum biochemistry using a CELL-DYN 3700 Hematology Analyzer (Abbott Laboratories, Abbott Park, IL), for serum Fe with a Hitachi 911 (Roche Laboratories, Indianapolis, IN), and vitamins A and E via the method of Weinmann et al. (*123*).

Metals and Dioxin Analysis for IPF

Priority metals and dioxins were analyzed for IPF by Columbia Analytical services in Kelso, WA and Houston, TX, respectively. As, Cd, Cr, Co, Pb, Mo, Ni, Se, and Zn were analyzed by EPA method 200.9, Hg was measured by EPA method 7471A, and Ba and Sr were analyzed by EPA method 6010B. Dioxins and furans were analyzed by EPA method 8280A.

Results and Discussion

Ferrihydrite at 0.5% w/w was found to reduce mean urinary arsenic (presented as mg As/mg urinary creatinine) levels from arsenite by 74.9% after 24 hours and 49.1% after 48 hours (Figure 40). For arsenate, we noted a 43.6% reduction in urinary arsenic after 24 hours and 39.5% after 48 hours (Figure 41). All arsenic reductions were found to be statistically significant ($p < 0.05$) except for arsenate at 24 hours. Ferrihydrite reduced total As in urine (not standardized for creatinine) from 1034.0 ppm to 305.1 ppm (70.5% reduction) for As(III) after 24 hours and from 86.1 ppm to 47.7 ppm (44.6%) after 48 hours (Figure 42). Ferrihydrite reduced urinary As from 701.0 ppm to 402.5 ppm (42.5%) after 24 hours and from 76.9 ppm to 43.2 ppm (43.8%) after 48 hours in experiments with As(V) (Figure 43). All reductions were statistically significant except for As(V) at 24 hours. This was due to the variability in As levels seen after 24 hours. These data show that ferrihydrite reduced biomarkers of arsenic exposure via drinking water when administered as an enterosorbent, and importantly, that ferrihydrite may work for both species of As.

For rats being fed 0.5% w/w ferrihydrite for 2 weeks, no statistically

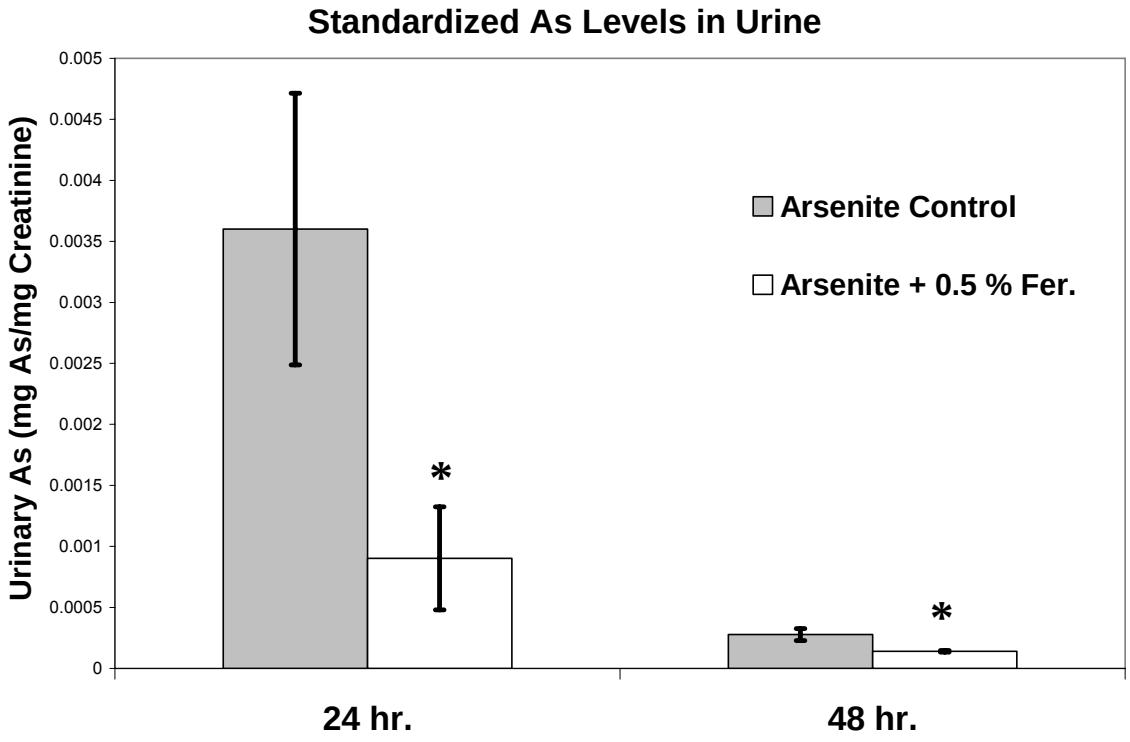

Figure 40. Reduction of standardized urinary As from arsenite by dietary ferrihydrite. Four week old male Sprague-Dawley rats were given one dose of 0.5 mL of 500 ppm arsenite with and without 0.5% w/w ferrihydrite by gavage and diet. Urine samples were collected at 24 and 48 hours and analyzed for total As and urinary creatinine. Urinary As values are reported as mean mg arsenic/mg creatinine ± 1 standard error.

* Indicates statistical significance ($p<0.05$) using the Wilcoxon Rank Sum Test when comparing between control (As only) and treated groups (As + 0.5 % ferrihydrite).

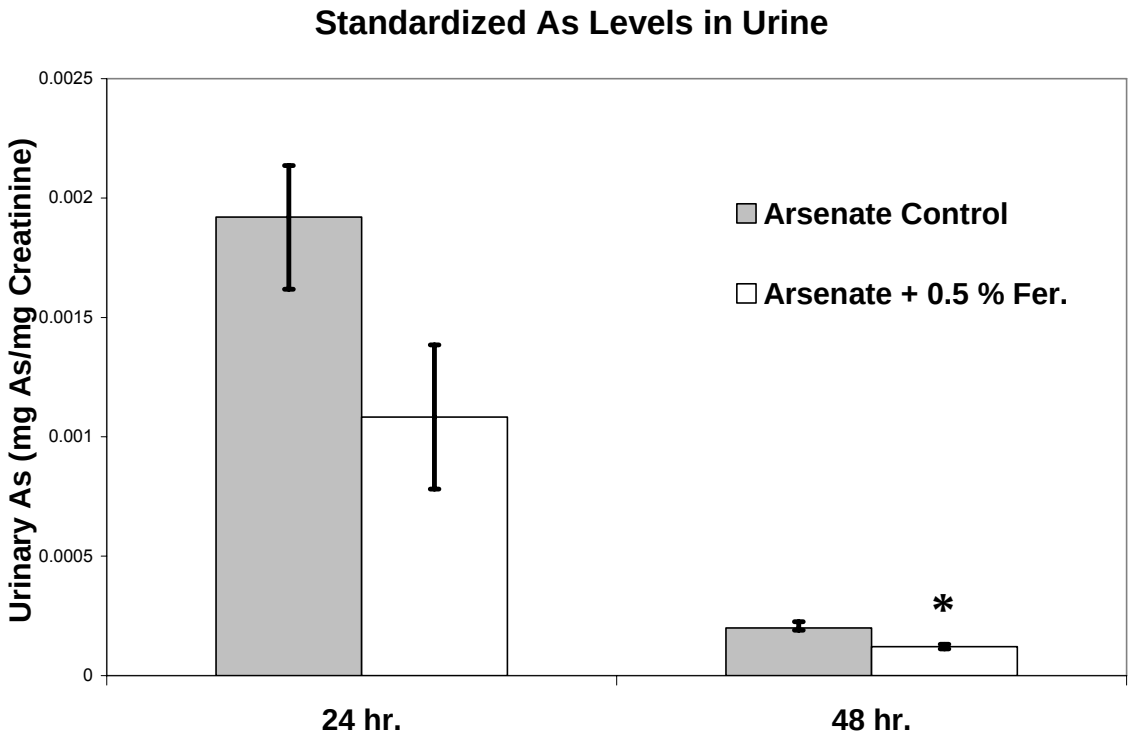

Figure 41. Reduction of standardized urinary As from arsenate by dietary ferrihydrite. Four week old male Sprague-Dawley rats were given one dose of 0.5 mL of 500 ppm arsenate with and without 0.5% w/w ferrihydrite by gavage and diet. Urine samples were collected at 24 and 48 hours and analyzed for total As and urinary creatinine. Urinary As values are reported as mean mg arsenic/mg creatinine ± 1 standard error.

* Indicates statistical significance ($p<0.05$) using the Wilcoxon Rank Sum Test when comparing between control (As only) and treated groups (As + 0.5 % ferrihydrite).

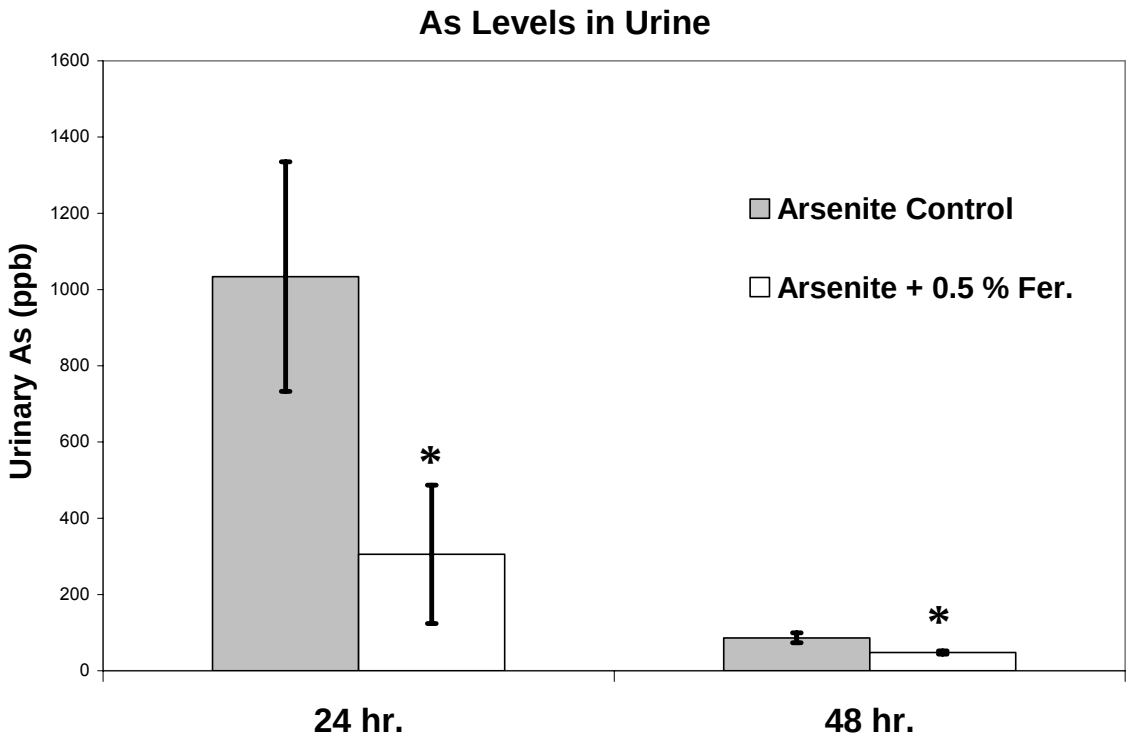

Figure 42. Reduction of unstandardized urinary As from arsenite by dietary ferrihydrite. Four week old male Sprague-Dawley rats were given one dose of 0.5 mL of 500 ppm arsenite or arsenate with and without 0.5% w/w ferrihydrite by gavage and diet. Urine samples were collected at 24 and 48 hours and analyzed for total As and urinary creatinine. Urinary As values are reported as mean mg arsenic/mg creatinine ± 1 standard error.

* Indicates statistical significance ($p<0.05$) using the Wilcoxon Rank Sum Test when comparing between control (As only) and treated groups (As + 0.5 % ferrihydrite).

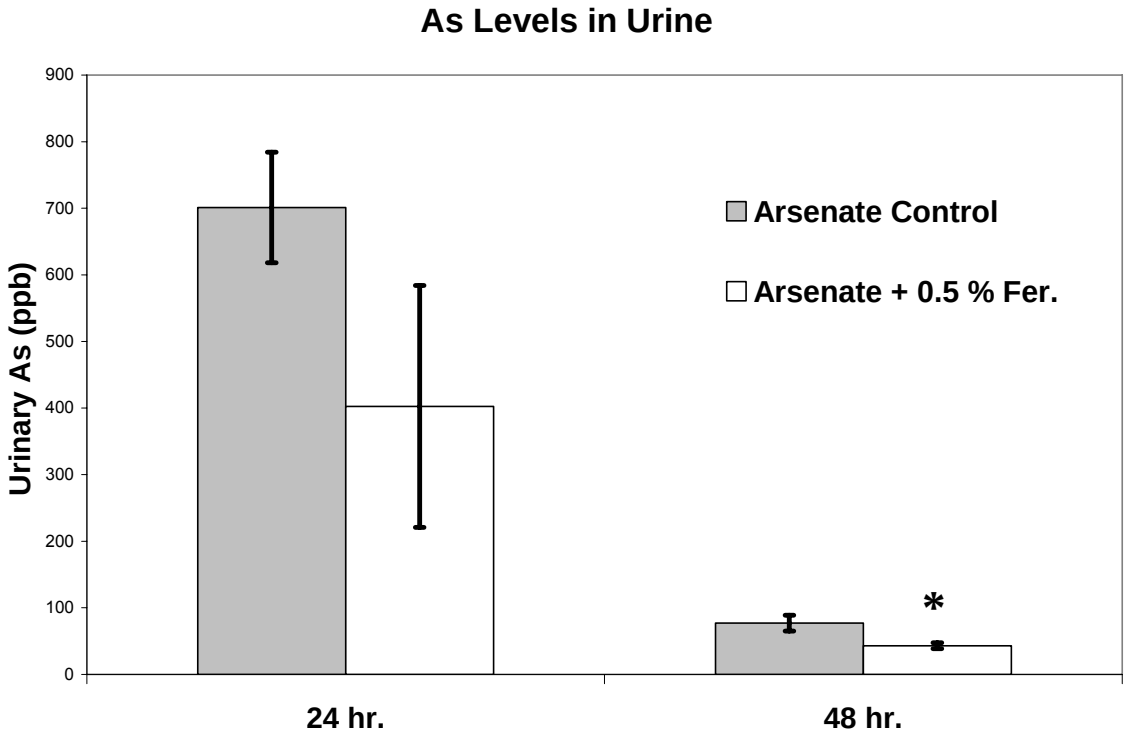

Figure 43. Reduction of standardized urinary As from arsenate by dietary ferrihydrite. Four week old male Sprague-Dawley rats were given one dose of 0.5 mL of 500 ppm arsenite or arsenate with and without 0.5% w/w ferrihydrite by gavage and diet. Urine samples were collected at 24 and 48 hours and analyzed for total As and urinary creatinine. Urinary As values are reported as mean mg arsenic/mg creatinine ± 1 standard error.

* Indicates statistical significance ($p<0.05$) using the Wilcoxon Rank Sum Test when comparing between control (As only) and treated groups (As + 0.5 % ferrihydrite).

significant differences were noted in serum biochemistry parameters, serum Fe, or vitamins A and E levels versus rats being fed a control diet (Table 11). All measured parameters were also within clinical reference ranges (*124*). No differences were seen in total feed consumed or feed conversion rates (g food eaten/g weight gained). All organs appeared normal at necropsy with no lesions being noted with animals consuming 0.5% ferrihydrite or control feed. Importantly, serum Fe levels were roughly the same in both groups which is encouraging considering that ferrihydrite does have the potential for acid dissolution in very low pH conditions such as those potentially seen in the stomach. Also notable was the lack of effect of ferrihydrite on serum phosphorus. Our results suggest that ferrihydrite at 0.5% w/w does not effect phosphate utilization after a period of 2 weeks. Further studies are warranted to confirm that ferrihydrite would not interfere with the utilization of important micronutrients such as phosphate, iron and zinc, specially in developing countries where high As exposure is prevalent along with malnutrition and infectious disease. Importantly, our data confirm previous work that the iron from ferrihydrite is not likely to be absorbed (*125*). Overall results suggest that ferrihydrite at 0.5% is well tolerated by the rats in our study, and that it is apparently safe for short term administration when given to healthy rats, well nourished rats.

Metal and dioxin analysis for IPF showed that priority metals, dioxins and furans were well below tolerable daily intakes suggested by JECFA and WHO (Tables 12-13). Interestingly, Fe levels in IPF were found to be 65.1% of the composition of IPF, which suggests other iron phases may be present in the

Table 11. Serum components tested and their respective units.

	Treatment Group	
	0.5% Ferrihydrite	Control
Total serum protein (g/dL)	6.0 ± 0.2	6.1 ± 0.2
albumin (g/dL)	4.15 ± 0.12	4.33 ± 0.19
calcium (mg/dL)	12.617 ± 0.454	12.200 ± 0.352
phosphorus (mg/dL)	12.948 ± 0.992	13.160 ± 0.905
glucose (mg/dL)	268.3 ± 30.1	250.5 ± 31.9
BUN (mg/dL)	15.433 ± 0.905	15.533 ± 1.678
Creatinine (mg/dL)	0.202 ± 0.042	0.207 ± 0.015
Total bilirubin (mg/dL)	0.100	0.100
ALP (U/l)	186.7 ± 23.4	182.5 ± 33.7
CK (U/l)	554.5 ± 213.5	389.3 ± 77.0
AST (SGOT) (U/l)	147.333 ± 45.807	122.5 ± 27.245
ALT (SGPT) (U/l)	65.3± 20.2	58.5 ± 12.4
Globulins (g/dL)	1.800 ± 0.126	1.767 ± 0.082
A/G Ratio	2.315 ± 0.184	2.457 ± 0.184
GGT (U/l)	<3	<3
Amylase (U/l)	2356.5 ± 317.6	2343.0 ± 360.9
Cholesterol (mg/dL)	100.083 ± 16.398	97.233 ± 7.292
Fe (µg/dL)	123.5 ± 41.6	114.3 ± 45.5
Vitamin A (ng/mL)	324.7 ± 188.3	310.9 ± 175.4
Vitamin E (µg/dL)	5.678 ± 0.734	5.590 ± 0.785
Total weight gain (g)	95.4 ± 5.4	92.4 ± 6.2
Feed Conversion Ratio	0.339 ± 0.019	0.337 ± 0.016

Values are reported ± one standard deviation.

To compare between treated groups (0.5% Ferrihydrite) and controls, the Wilcoxon Rank Sum Test was used. No statistically significant differences ($p < 0.05$) were noted throughout serum tests. N = 6 for both groups.

sample. From ferrihydrite's empirical formula of Fe_5HO_8* $4H_2O$, Fe would be expected to constitute approximately 58% of the sample, assuming no adsorbed water. Although not seen on the X-ray diffraction pattern, there could be small amounts of goethite (FeOOH), hematite (Fe_2O_3) or other iron forms with a higher % of Fe (Figures 44, 45). This could partially explain IPF's lower surface area versus laboratory produced ferrihydrite as goethite and hematite have lower surface areas than ferrihydrite (*50*).

These data confirm previous work in vitro and demonstrate the potential of ferrihydrite to serve as an enterosorbent for arsenic. From short term studies, ferrihydrite is apparently safe and highly effective as a treatment for arsenic. Although further studies are warranted, therapeutic applications for humans would likely consist of delivery of a few grams of ferrihydrite per day per individual. Enterosorption treatment with ferrihydrite or similar materials would represent an inexpensive strategy for individuals with limited access to clean water and to those traveling to areas with inadequate water remediation techniques. Future work will focus on the long-term safety and optimum dosage forms for ferrihdyrite.

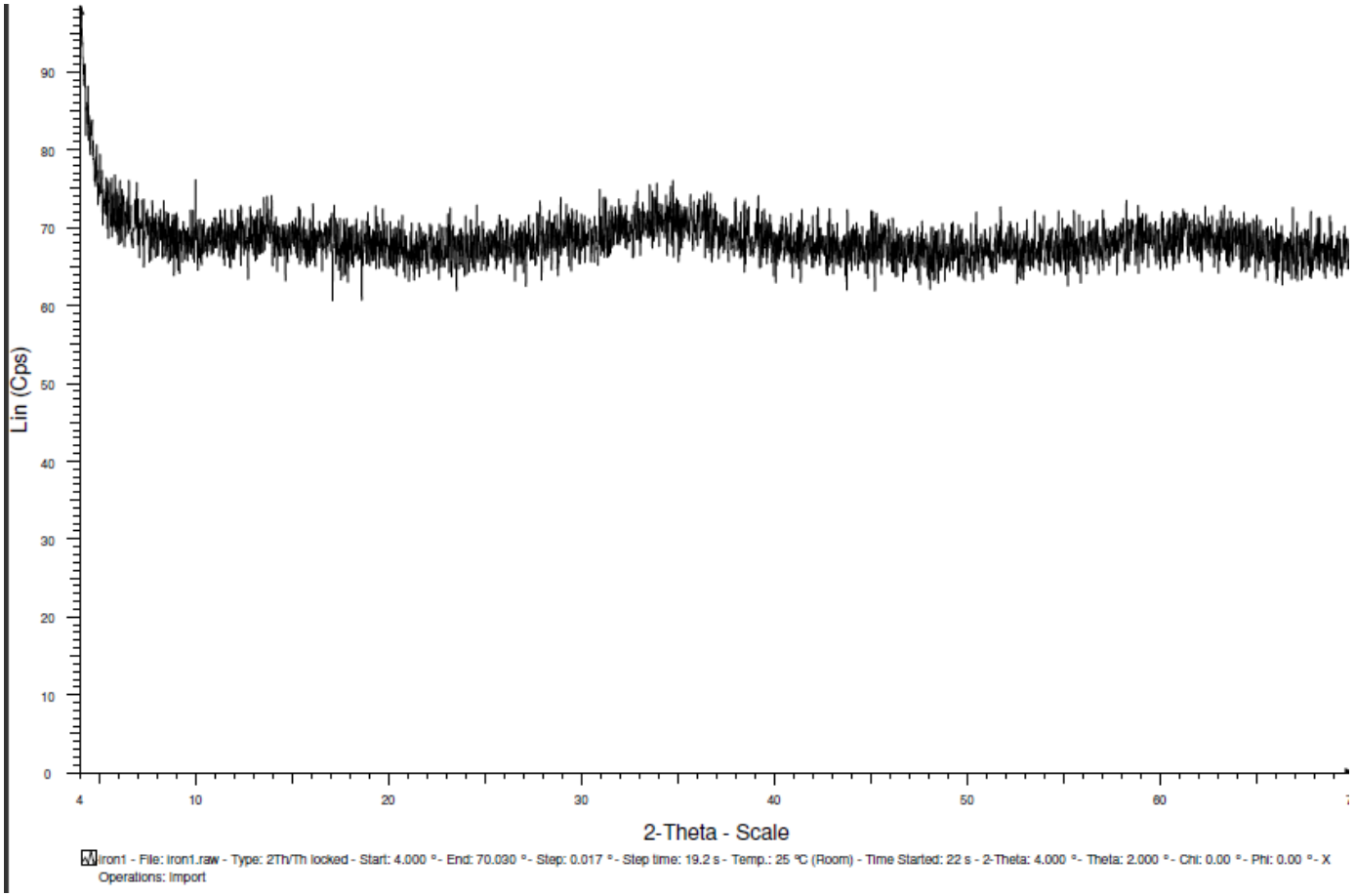

Figure 44. X-ray diffraction pattern for lab synthesized 2-line ferrihydrite. Results show a poorly crystalline material with 2 broad peaks confirming ferrihydrite in the sample.

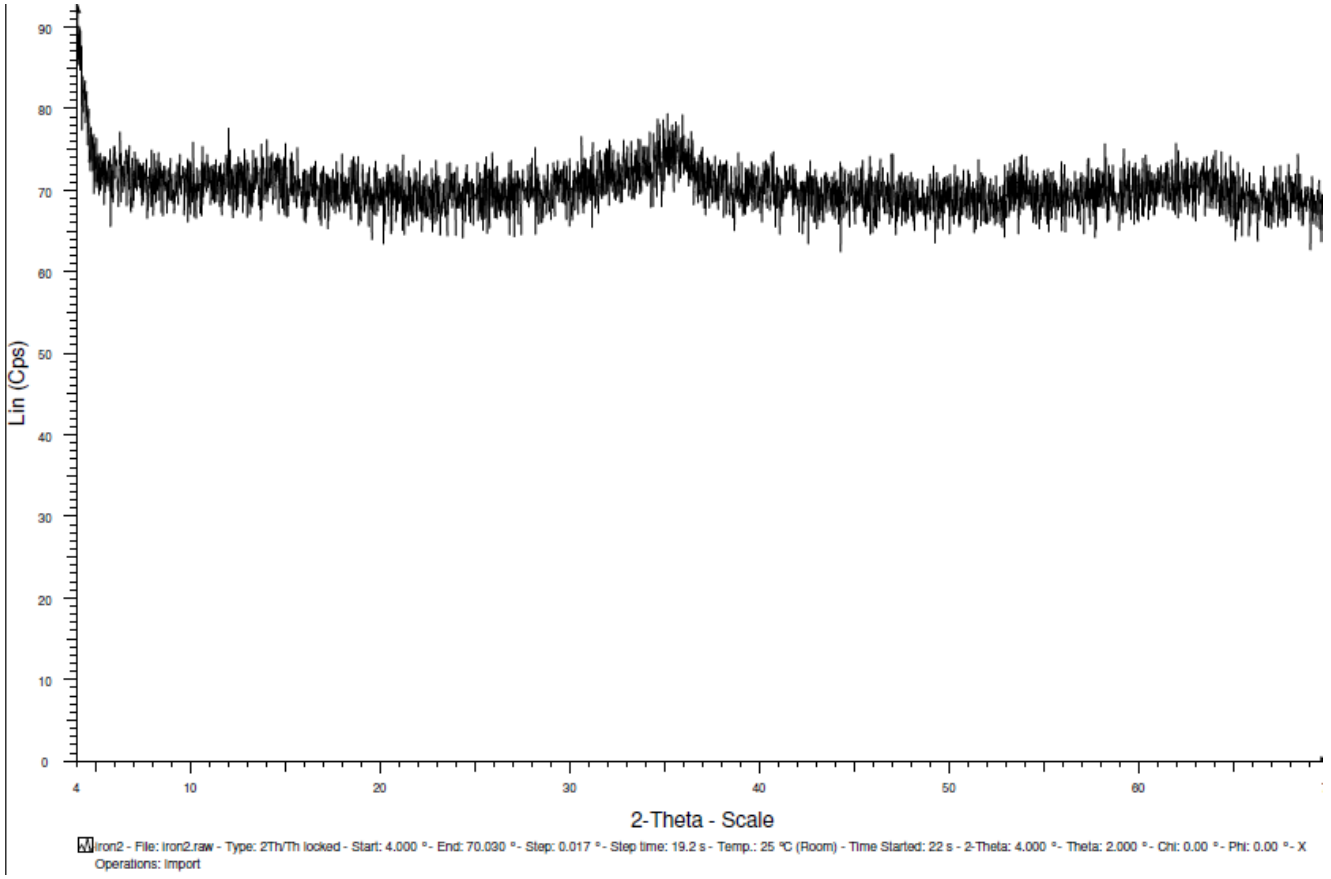

Figure 45. X-ray diffraction pattern for IPF. Results confirm that the material is 2-line ferrihydrite with slightly sharper peaks than for lab synthesized ferrihydrite, which means a slightly more crystalline ferrrihdyrite.

Table 12. Doixins and furans measured from IPF.

Dioxin or Furan	TEF[a] (WHO 2005)	IPF avg.
2,3,7,8-TCDD	1	ND
1,2,3,7,8-PeCDD	1	ND
1,2,3,4,7,8-HxCDD	0.1	ND
1,2,3,6,7,8-HxCDD	0.1	ND
1,2,3,7,8,9-HxCDD	0.1	0.0158000
1,2,3,4,6,7,8-HpCDD	0.01	0.0044500
OCDD	0.0003	0.0005860
2,3,7,8-TCDF	0.1	ND
1,2,3,7,8-PeCDF	0.03	ND
2,3,4,7,8-PeCDF	0.3	ND
1,2,3,4,7,8-HxCDF	0.1	ND
1,2,3,6,7,8-HxCDF	0.1	ND
1,2,3,7,8,9-HxCDF	0.1	ND
2,3,4,6,7,8-HxCDF	0.1	0.0104333
1,2,3,4,6,7,8-HpCDF	0.01	0.0035100
1,2,3,4,7,8,9-HpCDF	0.01	ND
OCDF	0.0003	0.0000571
Total TEF		0.03760977
	WHO TDI[b] (pg/kg/day)	pg/kg body weight in 3g/day IPF
	2.3	0.001611847

a TEF = Toxicity Equivalency Factor
b TDI = Tolerable Daily Intake
ND = None Detected

Table 13. Selected priority metals analyzed for IPF.

Metal	Conc. in IPF (ppm)	Amt. in 3g IPF (mg)	JECFA[a] TDI (mg/day)
As	0.4	0.0012	0.31
Ba	2.3	0.0069	3.57
Cd	0.167	0.000501	0.6
Co	2.3	0.0069	0.016
Cr	53.4	0.1602	0.25
Fe	651000	1953	NA
Hg	0.002	0.000006	0.043
Mo	3.76	0.01128	0.11
Ni	47.5	0.1425	0.3
Pb	4.81	0.01443	0.21
Se	1.3	0.0039	0.057
Sr	10.4	0.0312	5
Zn	19.8	0.0594	10

a JECFA = Joint FAO/WHO Expert Committee on Food Additives

CHAPTER V

SUMMARY AND CONCLUSIONS

Arsenic in drinking in water is a significant problem throughout the world. Chronic exposure leads to a variety of health effects including an increased incidence of cancer and characteristic skin lesions. Although the treatment of drinking water or finding alternate sources that are safe would be the best solutions to protecting exposed populations, these options are not always available or cost effective. This is especially the case since highly exposed individuals often occur in poorer countries. Currently, no enterosorbent has been shown to be effective in protecting individuals who are chronically exposed to As although chelating agents that contain sulfur groups have been proposed, but not confirmed (*11*).

The first goal of this research was to screen a variety of sorbents representing for their ability to bind arsenic as arsenite, As(III), and arsenate, As(V). It is important that the sorbent bind both As species since both occur in groundwater due to slow redox reactions of As and both are highly toxic. From the literature on As sorption, direct comparison of As binding and capacities of various sorbents is limited. Also here are conflicting reports, such as Mohan and Pittman, who suggested that goethite has a higher capacity for arsenite than ferrihydrite, which was not confirmed in our studies with several goethite sources (*126*).

The sorbents tested in screening assays included the phyllosilicates muscovite, halloysite, NovaSil (a calcium montmorillonite), SWy-2 (a sodium montmorillonite), attapulgite, the zeolite clinoptilolite, exchanged clays like CP-LPHM, SW-CYSTI, SW-CYSTE, and SW-THIAM, and finally the iron oxides goethite, magnetite, and two ferrihydrites. Reactive hydroxyls that are responsible for the sorption of As are found primarily on the iron oxides, particularly ferrihydrite, so they were expected to be the superior sorbents going into the experiments.

Synthetic ferrihydrite, produced in our laboratory or at BASF (IPF), were found to be the best sorbents of arsenic, both as As(III) and As(V). The exchanging of SWy-2 with organic compounds with sulfur moieties markedly increased the sorption of both As(III) and As(V) over raw SWy-2. Although these amended clays did not have the same capacity as ferrhydrite, this finding is important as these clays also sorb heavy metals such as mercury, which shows they are multi-functional sorbents. The clay minerals tested did not have a high enough capacity for As to be considered for further testing, but their inclusion was very important to the screening, as NovaSil and SWy-2 have both been shown to be safe in animal studies. Additional isotherms on vitamin A and riboflavin suggest that ferrihydrite and other soil minerals tested in this research will not interact with these important nutrients if given as an enterosorbent in the diet of animals or humans.

The next step in evaluating a possible enterosorbent for As was to further characterize the binding of As(III) and As(V) to ferrihydrite using isothermal analysis. Both sources offerrihydrite showed a high capacity for binding As(III) and As(V). The results showed that their toxin binding capacity was either equal to or greater than NovaSil for aflatoxins. Because these experiments were done at pH 7 and ferrihydrite's sorption of As(III) and As(V) are pH dependent, the next step was to evaluate ferrihydrite's sorption using a simulated gastrointestinal (GI) model.. Results from the simulated stomach (pH 1.8) revealed that both 2-line ferrihydrite and IPF showed similar capacities for As compared to aflatoxin sorbents. Moreover, As remained bound throughtout the simulated intestine. Our studies are the first to test ferrihydrite's binding ability for As at the pH encountered in the stomach after ingestion. The results showed that ferrihydrite bound both As species in a simulated GI model at levels which were correlated to efficacy in vivo with aflatoxin binders.

Another important measurement was Fe that was either liberated from acid dissolution from ferrihydrite or was small enough in particle size to pass through a 0.45 μm filter. From our studies, the estimated Fe (likely in the form of Fe^{3+}) was below the daily recommendation for Fe from ferrihdyrite and within tolerable limits for IPF. Thus, the total amount of iron that may be bioavailale was well below the tolerable daily intake in all of the experiments. Considering the As exposed, iron deficient population that this enterosorbent is intended for, any Fe supplementation that would occur from treatment with ferrihydrite would

likely be beneficial. Fe deficiencies have been noted as the single greatest nutrient deficiency worldwide. Also, since vitamin A helps mobilize Fe from storage, and these populations are also potentially deficient in vitamin A, iron supplementation from ferrihydrite could be therapeutic. Close monitoring, would however, be needed, as certain individuals are sensitive to Fe. These people store toxic levels of Fe, with severe disease resulting in a condition called hemachromatosis and the use of ferrihydrite may be contraindicated

The ability of ferrihydrite to protect a sensitive aquatic organism, *Hydra vulgaris* was also evaluated. Ferrihydrite was demonstrated to protect Hydra at high levels that were predicted from prior studies. The increased toxicity of As(III) versus As(V) was also confirmed using this assay supporting earlier work in animals and humans. The Hydra gut and exterior is in equilibrium with the surrounding solution making the Hydra assay a useful tool for the evaluation of toxicity of both As and the sorbent. No adverse effects were seen in Hydra exposed up to 0.5% ferrihydrite. Importantly, in the As(III) tests, ferrihydrite at 0.25% w/w showed that it would sorb over 99% of the As(III) in solution. It protected Hydra up to 200 ppm As whereas, 1 ppm was found to be lethal to the organism. This notable capacity is vital to ferrihydrite's success as an enterosorbent because certain individuals are highly exposed to As (ppm levels) in their drinking water. Since As(III) is the most toxic As form, and the most likely to be found in anoxic well water, it is important that ferrihydrite sorb a very high

percentage of As in solution. If binding is less efficient, then people chronically exposed to As could still exhibit increased cancer risks with enterosorbents.

The short term safety and efficacy of ferrihydrite was tested in vivo using a rodent model. Ferrihydrite added at 0.5% w/w in the diet was found to significantly reduce biomarkers of arsenic exposureThese results confirm that ferrihydrite is an effective enterosorbent for As from water in rodents. Also, 0.5% w/w ferrihydrite was apparently safe as evidenced by no statistically significant difference in body weights, feed conversion, serum biochemistry, and serum Fe. Importantly, no lesions were observed in in major organs upon necropsy. Also, no differences in serum Fe or serum phosphorus were found, indicating that ferrihydrite did not affect phosphate utilization. Certain individuals can be highly sensitive to iron and other dietary supplements, so any implementation of ferrihydrite as a dietary enterosorbent would need to be closely monitored when administered to a human population.

Altogether these data show that ferrihydrite has the potential to serve as an enterosorbent for As. ATSDR commented in their 2005 toxicological profile of As that “phosphate binders” needed to be researched as a specific binder for As (*11*). In this research, we have shown for the first time that ferrihydrite is apparently safe and effective in sequestering As in vitro, and in vivo in short term studies. Since there is no treatment (other than water filtration) currently available for chronic As exposure, Enterosorption therapy could benefit millions of people exposed to As on a daily basis. . Further studies are needed in other

mammalian species and for longer durations to confirm ferrihydrite's safety and efficacy. Twice the minimal effective dose of enterosorbent based on mycotoxin studies was tested, and further work is needed to titrate and verify the optimum dosimetry and delivery forms for ferrihydrite. Further studies are also needed to verify the effects of ferrihydrite on Fe status in both healthy and malnourished animal models. The results of these experiments represent a novel treatment that could enhance public health and greatly benefit susceptible populations at high risk for As exposure and As toxicosis.

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